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<u>Phrynosoma</u> Systematics, Comparative Reproductive Ecology, and Conservation of a Texas Native

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Phrynosoma Systematics, Comparative Reproductive Ecology, and Conservation of a Texas Native

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Dedication

In memory of John Treanor (1953- 2001) - you always carried the beat for us to dance to. Your dedication to music, positive outlook, and love of life inspired everyone to live life with passion.

Acknowledgments

Each Chapter of this dissertation was possible with the help of not one but many people. Acknowledgments are given in each section, but here I would like to extend my person gratitude and admiration to some dear friends who have really stuck it out and have given to this dissertation and my graduate experience immeasurable support. Andrew H. Price, through your cynicism and guidance, thank you for your friendship and forthrightness through many difficult hurdles. I could always count on you for anything. Oscar Flores Villela thank you for your generosity in and outside of Mexico, I could not have accomplished so much in Mexico without your help. Thank you for opening up your home to such a gringa, sharing it with me and supporting me both in the field and later after I returned home. C. Riley Nelson thank you so much for picking me out early in my graduate career to take under your wing and teach me how to teach others with energy, enthusiasm, humor, a true love of teaching, and compassion for others. You made the biggest impact on my professional development of all the faculty I interacted with. Monica Swartz, you have been in the trenches with me always even when thousands of miles separated us during your (and my) many excursions into wild places. You'll never know how inspiring you are. Many

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Phrynosoma Systematics, Comparative Reproductive Ecology, and Conservation of a Texas Native

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Ecologists use phylogenies as tools for understanding underlying evolutionary patterns and historical influence on species' biology, physiology, and behavior. Phylogenetic relationships of Phrynosoma, horned lizards, were analyzed using sequences that encode the genes cytochrome *b*, ND4, 12S rRNA and 16S rRNA, as well as morphological characters. Field work was conducted on Mexican Phrynosoma to collect basic ecological information such as locality where each species was found, seasonal and daily activity periods, and reproductive cycles. Evolution of viviparity in Phrynosoma was studied in the context of the cold-climate hypothesis using generated phylogenies and data collected from field and museum specimens and data in the literature. Viviparous species showed two distinct reproductive patterns that were more divergent between closely related taxa. Reproductive cycles of viviparous species suggested

this mode of reproduction is more labile and cycles may shift to enable sympatric and syntopic species to coexist. Viviparous species were found at significantly higher minimal and median (but not maximum) altitudes compared to oviparous species, suggesting viviparous species are restricted to higher elevations. No difference in latitude was found between oviparous and viviparous species. A strong phylogenetic component overshadowed differences in geographic range and altitude. Oviparous and viviparous clades separated early in the evolution of the genus and reproductive mode was maintained in each lineage. Reconstruction of a hypothetical ancestor suggested the ancestor of Phrynosoma inhabitated altitudes 1654 - 1747 m in north-central Mexico. Oviparous species invaded lower altitudes while viviparous species remained at higher altitudes. Conservation of Texas horned lizards (Phrynosoma cornutum) was studied at 100 localities in Texas. Records from over 3,000 museum specimens and a large survey of Texans were used to study range and abundance patterns. Lizard censuses at 100 localities confirmed anecdotal reports the species had declined significantly in eastern and central portions of Texas, while more stable populations still existed in south and west Texas. Land-use patterns reflected loss of P. cornutum populations that ocurred in the 1960s and 1970s. However, invasion of Solenopsis invicta and rise in pesticides used to kill all ants, including the lizards' preferred diet, Pogonomyrmex, has led to an overall decline in food resources where habitat may still be viable.

Table of Contents

List of Tables

List of Figures

Chapter One: Systematics of Phrynosoma inferred from mitochondrial genes and morphological characters.

Horned lizards, genus Phrynosoma, form a monophyletic group of thirteen species most closely related to sand lizards within Phrynosomatidae (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Reeder, 1995; Reeder and Wiens, 1996). Species occur in a variety of habitats ranging from southwestern Canada to Guatemala. Phrynosoma species display variation in ecological traits and have been widely studied in the United States. Understanding interspecific relationships is essential for testing evolutionary hypotheses about several interesting ecological characteristics such as mode of reproduction (Zamudio and Parra-Olea, 2000) and defensive blood squirting (Sherbrooke and Middendorf, 2001).

Four hypotheses have been proposed regarding Phrynosoma interspecific relationships. Reeve (1952) and Presch (1969) focused on osteological characters and shared characteristics between species. Montanucci (1987) published the first study to analyze phylogenetics in the genus using modern methods. Reeder and Montanucci (2001) made the first attempt to incorporate genetic data from mitochondrial 12S and 16S rRNA sequences in a phylogenetic analysis, though two taxa (P. braconnieri and P. douglasii sensu stricto) were omitted from their analysis. Zamudio et al. (1997) split P. douglasii sensu lato, into two species (P. douglasii and P. hernandesi) based on molecular evidence and supported by

morphological features. However, this taxonomic change was omitted in Reeder and Montanucci's (2001) systematic study. Although Reeder and Montanucci's (2001) study is the most complete, generic level, systematic study to date, it still leaves many unanswered questions regarding Phrynosoma species relationships. Their analysis resolved only three well-supported groups out of ten clades in their best estimate of phylogeny and omitted two taxa. The following analysis incorporates data from all species.

I analyzed mitochondrial ND4 and cytochrome *b* sequence data from all species of Phrynosoma, including P. braconnieri, P. douglasii (sensu stricto) and P. hernandesi. These data were also analyzed with the 12S and 16S rRNA sequence data and morphological data provided by Reeder and Montanucci (2001). Data sets were subjected to a variety of tests to determine how compatible or congruent they were. These tests were developed in response to debates regarding how to analyze multiple data sets; whether all data should be combined regardless of differences in data structure, all data should be analyzed separately or only those data sets that are congruent or compatible should be combined in a single analysis (Bull et al. 1993; Chippindale and Wiens, 1994; de Queiroz et al. 1995; Miyamoto and Fitch, 1995; Huelsenbeck et al. 1996). Rather than adhere to a dogmatic stance a priori, I chose a more pragmatic method of using all available diagnostics from both approaches to investigate the data. While pointing to conflicts in analyses of individual data sets, results from these tests were also used to investigate the nature of conflict between different data sets in combined analyses.

Data were first tested for phylogenetic signal using g_1 (skewness) statistics and permutation tail probabilities. Next, data were tested for combinability using the partition homogeneity test: results were mixed and indicated molecular data partitions could be combined while morphology should not be combined. Data sets were analyzed separately and the molecular data combined; consensus trees for each data set were produced to show areas of agreement and strong support using different phylogenetic methods. Taxonomic congruence between topologies from each data set and an alternate topology where all viviparous species were placed in a monophyletic group were tested using Wilcoxon signed-rank (Templeton) tests, winning-sites tests and parametric bootstrap (Monte Carlo) simulations. Taxonomic congruence tests produced conflicting results making it unclear whether molecular data were congruent. Data were combined in an attempt to gain further understanding about where incongruence or conflict may occur between data sets.

Partitioned Bremer indices were used to analyze the combined data to determine if conflict in the data could be attributed to certain relationships or portions of the topology. Skewness (g_1) statistics were used again to study the behavior of the data when different taxa were deleted and topologies constrained. When members of strongly supported clades were removed from analyses, the molecular data became randomized suggesting phylogenetic signal was only present in certain parts of the overall Phrynosoma topology. Analyses using multiple techniques and multiple methods provided clear evidence and strong support for relationships between half the clades in the genus.

MATERIALS AND METHODS

Specimen Collection

Specimens and tissue samples for all Mexican Phrynosoma species were collected in 1998 and 1999. Liver tissue from one P. mcallii specimen collected (by WLH) in 1994 during fieldwork conducted in Yuma, Arizona, was used to extract DNA for both cytochrome *b* and ND4 polymerase chain reactions (PCRs). ND4 was later amplified from Reeder's original P. mcallii sample. Three P. douglasii DNA samples were obtained (by KRZ) during fieldwork in 1990-1993. Extracted DNA from other Phrynosoma species was obtained from T. Reeder. Multiple specimens for several species were used in sequencing, but after initial phylogenetic analyses showed consistent conspecific pairing, duplicates were dropped and samples were matched across all genetic analyses so a single specimen for each species was used when possible. By reducing the number of taxa, maximum likelihood analyses went much quicker. Tissues were collected for P. cerroense, but only cytochrome *b* was sequenced. The validity of P. cerroense as a unique species has been argued, but verifying species status is beyond the scope of this work (Jennings, 1988; Grismer and Mellink, 1994; Brattstrom, 1997). Phrynosoma cerroense was not included in the primary analysis because little data were available and preliminary analysis of cytochrome *b* sequences suggested this species would pair with P. coronatum and not provide additional information regarding overall interspecific relationships.

Outgroup taxa included Callisaurus draconoides, Cophosaurus texanus, Holbrookia maculata, Sceloporus merriami, Urosaurus ornatus, and Uta

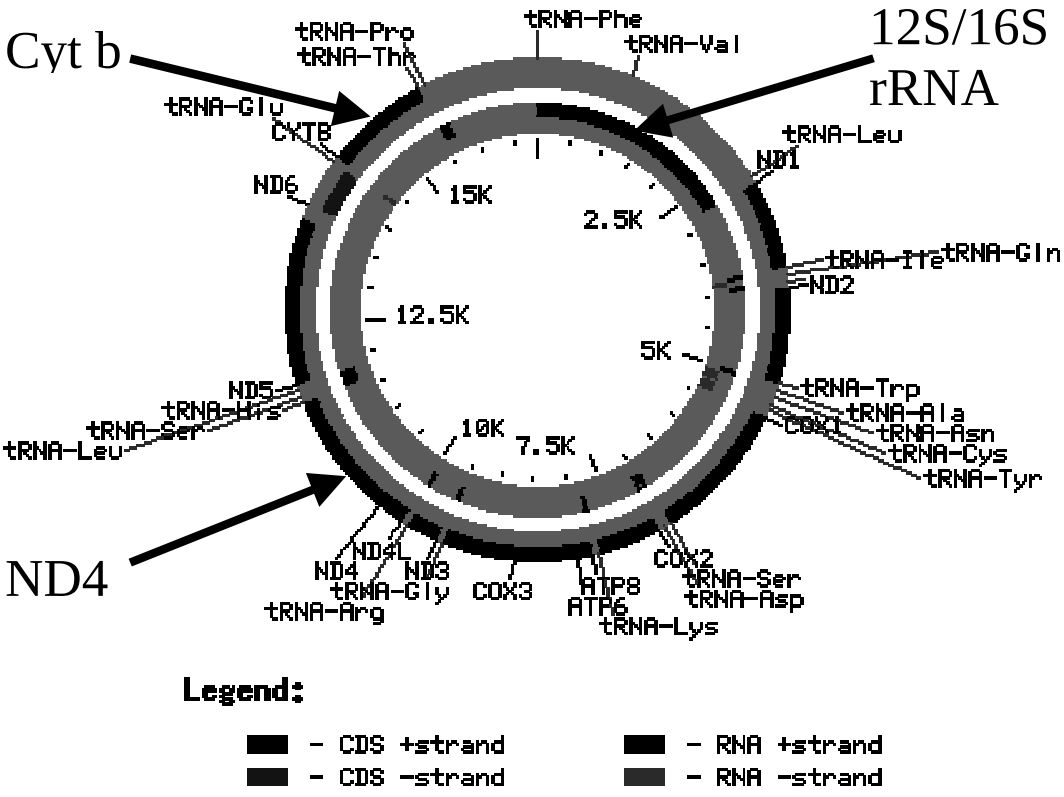
stansburiana (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989; Reeder, 1995; Reeder and Wiens, 1996). Extracted DNA samples for outgroup taxa were obtained from T. Reeder. Additionally, 12S and 16S rRNA sequences for all taxa except P. douglasii (sensu stricto) and P. braconnieri were downloaded from GenBank at the National Center for Biotechnology Information website. See Appendix 1 for specimen identifications and GenBank sequence submission numbers. Morphological data were downloaded from the website published in Reeder and Montanucci (2001). The morphological data do not distinguish characters assigned to either P. douglasii or P. hernandesi. Morphological data represent P. douglasii sensu lato because characters from specimens representing both P. douglasii and P. hernandesi were scored and the data pooled in the data matrix (Montanucci, personal communication). See Montanucci (1987) for the list specimens examined for the morphological data.

DNA Extraction, Amplification, Sequencing and Sequence Alignment

Purified and dried DNA samples obtained from T. Reeder were rehydrated in double distilled sterile water to use in PCRs. Other DNA samples were extracted using standard Chelex and phenol-chloroform extraction methods from liver tissue samples (Walsh et al., 1991; Hillis et al., 1996). Primers for cytochrome *b* follow published primers in Trépanier and Murphy (2001). Primers amplified the entire cytochrome *b* sequence and part of the tRNA-Thr, which was removed from the analysis later: 1044 nucleotides were analyzed from the cytochrome *b* gene. Primers used to amplify ND4 follow Zamudio et al. (1997), and a 753-base-pair segment of ND4 with tRNA-His and part of tRNA-Ser was

Figure 1: Whole Chrysemys picta Mitochondrial Genome.

Mitochondrial genome for Chrysemys picta illustrating placement of different genes. The entire cytochrome *b* gene (1044 nucleotides) was sequenced in this study along with the last half of ND4, tRNA-His and part of tRNA-Ser (753 nucleotides). Partial sequences for 12S and 16S rRNA (total 731 nucleotides) were downloaded from GenBank. This image is taken from the NCBI website (http://www.ncbi.nlm.nih.gov).



used in the analysis. Sequence data for all taxa from the 12S and 16S rRNA regions were downloaded from GenBank, adding 713 characters. A total of 2510 nucleotides was analyzed (Figure 1). Polymerase chain reactions were set up using 25 µl volumes of 8 µl DNA, 11.38 µl sterile, double distilled water, 2.5 µl

reaction buffer, 1.25 µl of each primer, 0.5 µl of dNTP mix, and 0.125 µl Taq polymerase. Samples were placed in 0.5 ml reaction tubes in a thermocycler under the following cycles: 94° C for 5 minutes (one cycle): 94° C for 1 minute, 45° C for 45 seconds, 72° C for 1 minute (30 cycles): final extension at 72° C for 5 minutes (1 cycle). Product was confirmed by running each PCR product on a 0.8% agarose gel in a Tris-acetate buffer for 15-30 minutes at 90-100 volts. Each PCR sample was purified from a 1.5% agarose gel using a QIA-quick gel extraction kit (No. 28704) following manufacturer's instructions. Most cytochrome *b* samples were reamplified and repurified from gel-purified products, yielding clean product for use in subsequent terminator sequencing reactions. Terminator sequence reactions consisted of 2 µl terminator mix (University of Texas at Austin Institute for Cellular and Molecular Biology), 1 µl primer, 1 µl 5X reaction buffer, 1-6 µl PCR products and enough sterile, double distilled water to bring reactions to 10 µl. Reaction sequence cycles were: 96° C for 10 seconds, 50° C for 5 seconds, 60° C for 4 minutes. Centri-Sep columns (Princeton Separations) were used to clean reactions and remove excess dyes prior to sequencing. Labeled sequences were run on an ABI Prism 377 DNA Sequencer.

Cytochrome *b* sequences were originally aligned to published sequences at GenBank for Alligator mississippiensis (Accession Number: NC_001922) and Chrysemys picta (Accession Number: NC_002073) because completely mapped genomes were available for these species; alignments were secondarily compared to Trépanier and Murphy (2001) Uma sequences available at GenBank. Alignments for ND4 were made against published sequences in Arevalo et al.

(1994) using Se-Al (version 2.0a6, Rambaut, 2001). Cytochrome *b* and ND4 (not the downstream tRNAs) alignments were both rechecked by converting nucleotides to amino acid sequences and checking for nonsense mutations. 12S and 16S rRNA sequences downloaded from GenBank were aligned using Clustal W (Thompson et al., 1994) under varying gap costs as described in Reeder and Montanucci (2001). Regions that aligned differently under different gap costs were considered ambiguous and excluded from phylogenetic analyses. Fifty-nine characters were excluded using this criterion. Final 12S and 16S rRNA alignments were compared to the data matrix provided by T. Reeder.

Phylogenetic, Combinability and Congruency Analyses

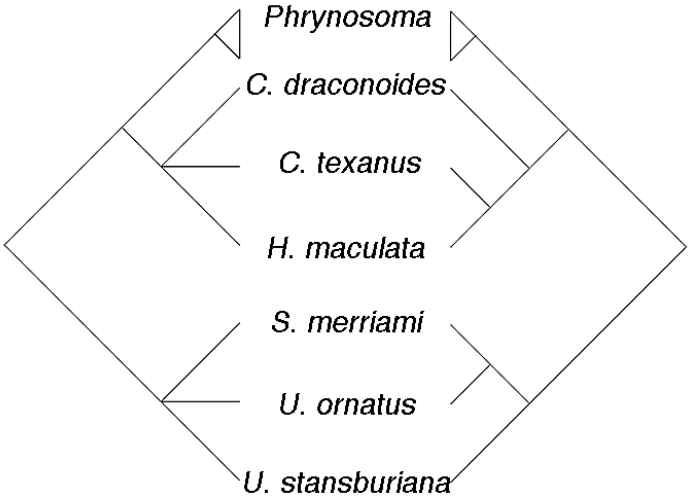
Phylogenetic structure was first assessed using the skewness statistic (g_1) from 10^6 random trees and permutation tail probability test in PAUP*. Skewness (g_1) values were compared to tables in Hillis and Huelsenbeck (1992). The partition homogeneity test in PAUP* was used to test whether different characters could be combined at the level of character type (nucleotide versus morphology), gene (12S/16S, cytochrome *b*, ND4), and codon position (1^{st}, 2^{nd}, 3^{rd}). All phylogenetic analyses were conducted using the outgroup comparison method (Watrous and Wheeler, 1981). Analyses were constrained by a nested relationship of the outgroup taxa relative to the ingroup (Figure 2): the sand lizard clade (Callisaurus, Cophosaurus, Holbrookia) was constrained to be most closely related to Phrynosoma, and Sceloporus, Urosaurus, and Uta were grouped outside of the Phrynosoma – sand lizard clade based on previous phylogenetic studies on Phrynosomatidae (Etheridge and de Queiroz, 1988; Frost and Etheridge, 1989;

Reeder, 1995; Reeder and Wiens, 1996). Reeder and Montanucci (2001) analyzed their data by constructing a hypothetical ancestor and using this ancestor to root their tree. Because they constructed ancestral characters from different outgroup taxa than this study, a new ancestor using the outgroup taxa in this study was constructed in MacClade (Version 3.08a, Maddison and Maddison, 1992) following the algorithm of Maddison et al. (1984). This allowed us to use all of Reeder and Montanucci's (2001) data in morphological analyses. All analyses using genetic data were conducted using both original outgroup data and data computed for the hypothetical ancestor. No difference in results should arise from using outgroups versus a computed ancestor; however, using a computed ancestor could find locally optimal parsimonious trees as opposed to a globally parsimonious tree found when using outgroup taxa themselves (Maddison et al., 1984). This problem can be averted if the outgroup relationships are well resolved. When reconstructing states for a hypothetical ancestor, I used the topology from Etheridge and de Queiroz (1988), corroborated by Reeder and Wiens (1996), as the most resolved topology for the outgroups (Figure 2).

Parsimony and maximum likelihood analyses were conducted on molecular data with PAUP* (version 4.0b8a, Swofford, 2001) using the heuristic search option with 1000 random addition sequences to reduce chances of finding local instead of global optima (Swofford et al., 1996). Only parsimony analyses were performed when morphological characters were analyzed. The branch-and-bound option was attempted in all parsimony analyses, but this option often failed to make any progress after several days of running even for a single gene with 14

Figure 2: Topological Constraints.

Left side represents the nested relationship between outgroups used when analyzing data with all taxa included. Right side represents the most resolved relationships between outgroup taxa used to reconstruct ancestral characters.



taxa. When successfully implemented, branch-and-bound searches produced the same topologies found by heuristic searches. Initial upper bounds were computed by stepwise addition of taxa, and gaps were treated as missing characters. Data partitions for the initial parsimony analyses were unweighted and results were used to assess nucleotide base substitution rates at different codon positions and transition and transversion rates.

Nucleotide substitution rates and transition and transversion rates at different codon positions were first assessed using MEGA (version 2.1, Kumar et al., 2001). Uncorrected pairwise sequence (p) distances and Tamura-Nei distances were calculated and plotted for each pair of taxa. Uncorrected distances represent proportion of sites that are different between sequences, but do not

account for changes or biases in substitution rates. Tamura-Nei distances correct for multiple hits at sites taking into account substitutional rate differences and inequality of nucleotide frequencies (Tamura and Nei, 1993). If no difference in rates was present, points would fall along an isometric line. However, deviations from isometric lines qualitatively indicate changes are present in substitution rates and may suggest sites at certain pairwise distances are becoming saturated with change (Zamudio et al., 1997; Dowton and Austin, 2002). Deviations were seen in distance plots for different classes of nucleotides in both cytochrome *b* and ND4 data sets suggesting differences in rates of substitutions existed (Figures 3 and 4).

Substitution rates were estimated for each codon position, transitions, and transversions in both cytochrome *b* and ND4 data sets. Numbers of nucleotide changes at each codon position, transitions and transversions were generated using MacClade (Table 1). Rates of change were calculated by dividing the number of changes at a position by number of nucleotide bases sequenced. Each rate was standardized by dividing it by the lowest observed rate among all codon positions (Table 2). Rate information was used to assign weights to two classes of data (codon position and transition to transversion ratio), and weighted parsimony analyses were performed. Codon positions were weighted by dividing the highest rate among all three positions by the rate at each position, and transitions and transversions were weighted inversely with respect to the transition to transversion ratio. Parsimony analyses of the cytochrome *b*, and ND4 data were conducted under the same parameters as unweighted parsimony except for the assigned weights.

Figure 3: Pairwise Distance Plots for Cytochrome *b* .

Plots of uncorrected pairwise sequence (p) differences and Tamura-Nei differences for cytochrome *b* and computed ancestor. Plots from top to bottom represent codon position. Left plots are transitions and right plots are transversions. Isometric lines are shown for reference: deviations from this line are indications of multiple hits and differences in substitution rates at different sites.

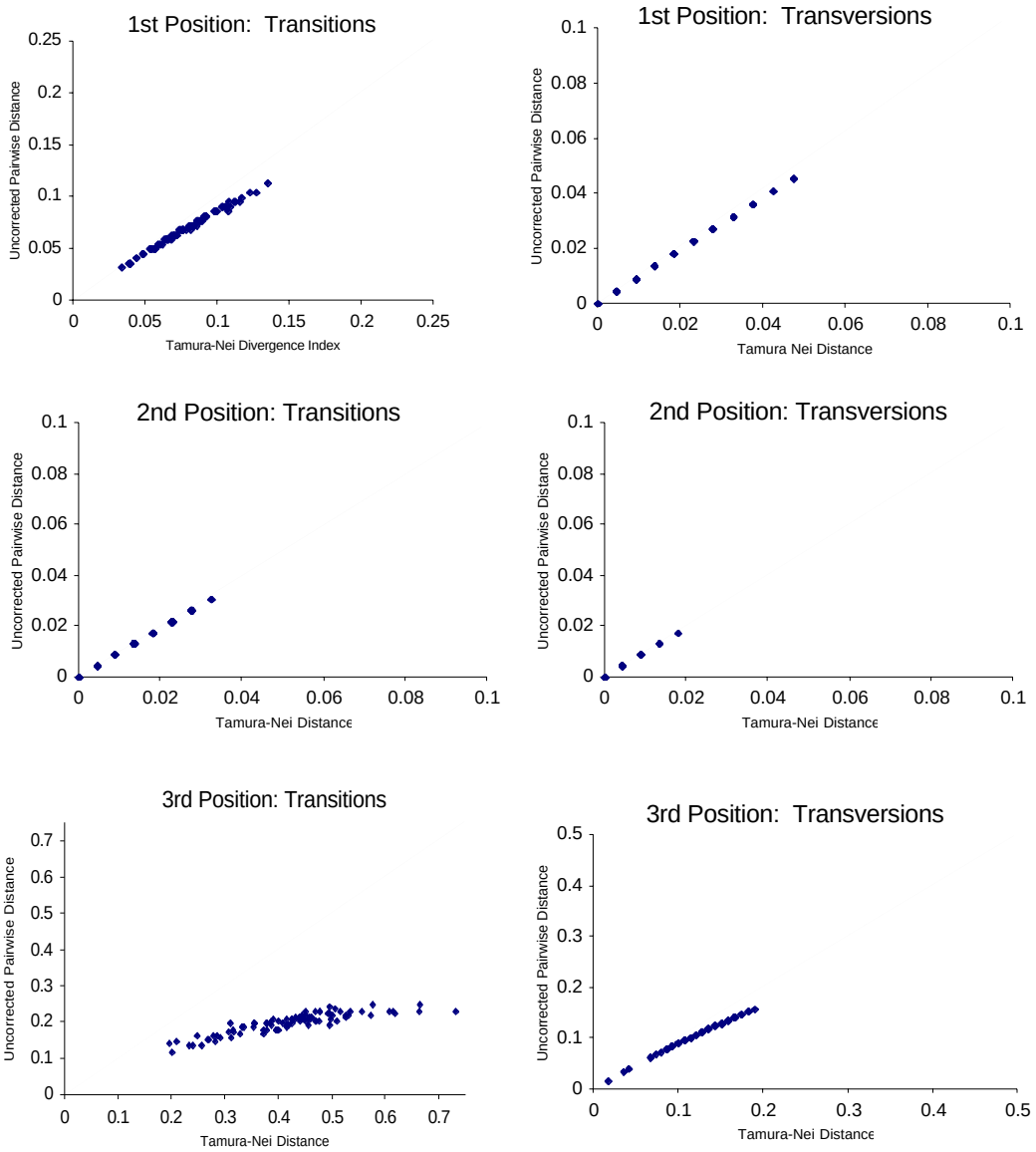


Figure 4: Pairwise Distance Plots at Each Codon Position for ND4 Transitions and Transversions.

Plots of uncorrected pairwise sequence (p) differences and Tamura-Nei differences for ND4 using the computed ancestor. Plots from top to bottom represent codon position. Left plots are transitions and right plots are transversions. Isometric lines are shown for reference: deviations from this line are indications of multiple hits and differences in substitution rates at different sites.

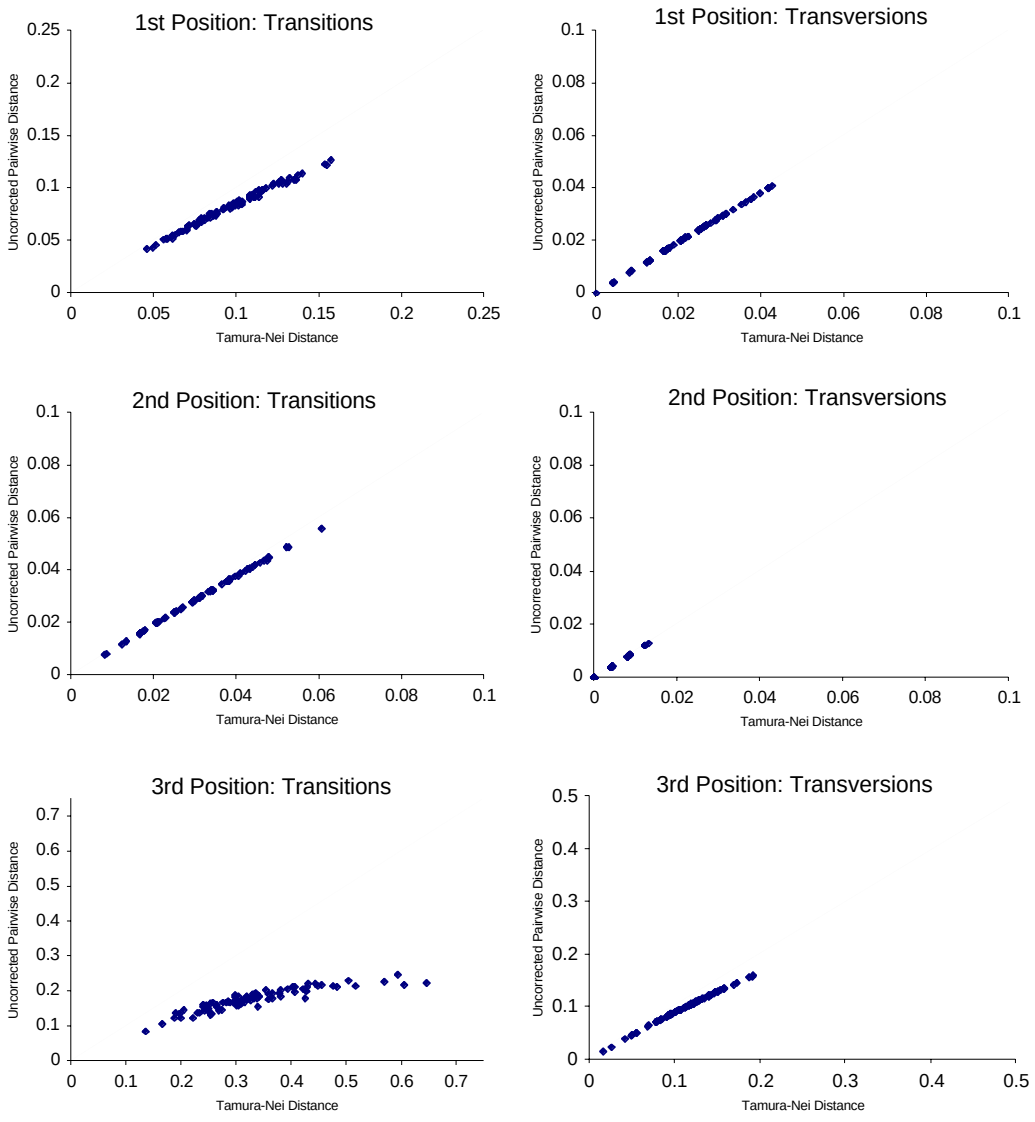


Table 1: Nucleotide Changes at Codon Positions, Transitions, and Transversions.

Numbers of changes at each codon position, transitions (Ti) and transversions (Tv), and Ti/Tv, generated from parsimony trees for cytochrome *b* and ND4 using MacClade. Data represent ranges for all trees found in each analysis. Ancestor refers to analyses using the computed ancestor as the outgroup. Outgroups refer to analyses using individual outgroup taxa.

Data Set / Outgroup	Cyt b Ancestor	Cyt b Outgroup	ND4 Ancestor	ND4 Outgroup
Total No. Characters in Each Sequence	1044	1044	753	753
No. Bases Sequenced at Each Codon Position	348	348	251	251
1st Position Changes	269-272	402-405	186-189	311-312
2nd Position Changes	67-69	92-96	55-55	94-96
3rd Position Changes	828-829	1324-1325	500-503	848
Transitions (Ti)	562-579	788-808	386-392	563
Transversions(Tv)	219-222	407-413	136-145	283
Ti/Tv Ratio	2.57-2.61	1.94-1.96	2.70-2.84	1.99

Table 2: Nucleotide Rates of Change and Weights.

Substitution rates for each codon position were determined by dividing observed maximum number of changes from unweighted, maximum parsimony analysis by total number of bases in each position and multiplying by 100. Each rate was standardized by the minimum rate calculated overall. Rates for data sets with a computed ancestor were calculated separately from outgroup taxa. Rates were converted to a weight by dividing the highest rate for each gene partition by individual codon position rates.

	Codon Position		
Gene Partition	**1**	**2**	**3**
Cyt b, Ancestor			
No. Changes (C)	272	69	829
No. bases sequenced (S)	348	348	348
Rate (C/S x 100)	78.2	19.8	238.2
Standard Rate	3.9	1.0	12.0
Assigned Weight	3	12	1
Cyt b, Outgroups			
No. Changes (C)	405	96	1325
No. bases sequenced (S)	348	348	348
Rate (C/S x 100)	116.4	27.6	380.7
Standard Rate	5.9	1.4	19.2
Assigned Weight	3	14	1
ND4, Ancestor			
No. Changes (C)	189	55	503
No. bases sequenced (S)	251	251	251
Rate (C/S x 100)	75.3	21.9	200.4
Standard Rate	3.8	1.1	10.1
Assigned Weight	3	9	1
ND4, Outgroups			
No. Changes (C)	312	96	848
No. bases sequenced (S)	251	251	251
Rate (C/S x 100)	124.3	38.2	337.8
Standard Rate	6.3	1.9	17.1
Assigned Weight	3	9	1

General maximum likelihood settings were the same as parsimony settings (e.g. heuristic search, gaps treated as missing characters), but TBR branch swapping and substitution models were also stipulated. Modeltest (version 3.06, Posada and Crandall, 1998) was used to determine the substitution model for each individual data set and combined DNA data. Modeltest chose TrN+I+Γ model of substitution as the best model for all genetic data sets, and the following parameters were incorporated into maximum likelihood analyses (Table 3): unequal base frequencies with rate matrices for each base in each data partition were calculated, among site rate variation was characterized with a gamma distribution with alpha (the shape parameter) calculated for each gene, and proportion of invariable sites was also calculated.

Table 3: Modeltest Parameters and Maximum Likelihood Scores.

Estimated parameters by Modeltest were used in maximum likelihood (ML) analyses. Best likelihood scores found by ML are also given. Ancestor refers to analyses using the computed ancestor as the outgroup, and Outgroups refer to analyses using individual outgroup taxa.

Data Partition	Cyt b Ancestor	Cyt b Outgroups	ND4 Ancestor	ND4 Outgroups
Total No. Sites in Analysis	1044	1044	753	753
Shape Parameter (alpha)	1.3932	1.3905	2.1885	1.536
Proportion of Invariable Sites	0.4962	0.4809	0.5495	0.494
Likelihood Score	6022.6037	8323.4783	4053.8356	5750

Data Partition	12S/16S Ancestor	12S/16S Outgroups	Combined mtDNA Ancestor	Combined mtDNA Outgroups
Total No. Sites in Analysis	669	669	2451	2451
Shape Parameter (alpha)	0.7099	0.3297	1.3302	1.1155
Proportion of Invariable Sites	0.6411	0.4378	0.5653	0.5202
Likelihood Score	1976.91	2725.385	12128.151	16774.632

Clade support was assessed using non-parametric bootstrap values and decay indices (Felsenstein, 1985; Bremer, 1994). One hundred bootstrap replicates with 1000 random addition sequences were performed in parsimony analyses and 100 bootstrap replicates for 10-1000 random addition sequences (depending on the ability of PAUP* to handle the data) were performed in maximum likelihood analyses. Decay indices (Bremer support) and partitioned decay indices (partitioned Bremer support) were calculated using TreeRot (version 2, Sorenson, 1999). Decay indices were used to assess relative contribution of different data partitions for a given clade on combined data phylogenies (Bremer, 1994; Baker et al., 1998). Partitioned decay indices were also used in combined analysis to explore conflicts between different data sets.

Taxonomic Congruence and Alternate Topology Tests

Consensus trees were produced showing congruence between parsimony and maximum likelihood analyses of each data set. Taxonomic congruence from separate analyses of each gene, all combined genes, morphological data, and all combined data was assessed using Wilcoxon signed-rank, two-tailed tests (Templeton, 1983) and winning-sites test in PAUP*. Pruning was necessary in some instances because taxa differed between comparisons. For example, outgroup taxa in comparisons between computed ancestor and outgroups were pruned before analyzing ingroup topological congruence. Tests for taxonomic congruence and alternate topologies were also performed using Monte Carlo (parametric bootstrap) simulations described in Goldman et al. (2000). Topologies from alternate data sets were designated as null hypotheses. A test

statistic was calculated based on differences between tree scores from the topology produced in the original parsimony analysis and the null topology used to constrain parsimony analysis with the same data set. One hundred data sets were generated by Monte Carlo simulation in Seq-Gen (version 1.2.5, Rambaut and Grassly, 1997). Model parameters used to simulate the data sets were estimated by PAUP* under a GTR + I + Γ model of evolution (the model of evolution was chosen based on nested likelihood ratio tests). Using parsimony optimization in PAUP*, tree length scores were generated from the simulated data and used to create a null distribution of differences in scores between topologies. For example, using cytochrome *b* data, the shortest tree was found under parsimony criteria and the topology from maximum likelihood analysis of the same data was used as a null hypothesis. Other topological tests included a tree where all viviparous species were monophyletic, trees produced in parsimony and maximum likelihood analyses of ND4 data and 12S/16S rRNA, parsimony analyses of morphological data, parsimony and maximum likelihood analyses of all genetic data combined and parsimony analysis of all data (genetic and morphological). Similar tests were performed using the ND4 data set.

RESULTS

A total of 2,542 characters was available for analyses. Fifty-nine characters were excluded because of ambiguous alignment, leaving 2,483 total characters (2,451 nucleotides and 32 morphological characters). Outgroup analyses (molecular data only) included a total of 976 variable sites and 719 informative sites. Combined characters (all molecular and morphological data)

with a constructed ancestor included 858 variable sites and 590 informative sites (see Table 4 for information on each data partition). All genes displayed unequal base frequencies with low guanine content and high adenine or thymine content.

Table 4: Patterns of Variation and Summary Statistics from Parsimony Analysis.

Gene partitions (cytochrome *b*, ND4, 12S/16S rRNA, combined mtDNA) were analyzed by rooting the ingroup with a computed ancestor (Ancestor) or with individual outgroup taxa (Outgroups). All Data Combined includes all mitochondrial genes and morphological data. No morphological data were available for outgroup taxa, so only the computed ancestor was used to root the tree during analyses with all data combined.

Data Partition	Cytochrome b Ancestor	Cytochrome b Outgroups	ND4 Ancestor
Number of Taxa	14	19	14
Total No. Sites in Analysis	1044	1044	753
No. Variable Sites (V)	430	484	290
No. informative Sites (I)	303	374	200
Ratio I/V	0.7047	0.7727	0.6897
%A (mean)	0.2982	0.2983	0.3495
%C (mean)	0.2641	0.2638	0.2602
%G (mean)	0.1262	0.1270	0.1108
%T (mean)	0.3115	0.3109	0.2796
No. Trees	2	2	3
Tree Length	1167	1822	744
Consistency Index (CI)	0.4876	0.4012	0.5134
Retention Index (RI)	0.3421	0.3299	0.3780
Rescaled Consistency Index (RC)	0.1668	0.1323	0.1941
Homoplasy Index (HI)	0.5124	0.5988	0.4866
CI excluding uninformative	0.4114	0.3502	0.4353
HI excluding uninformative	0.5886	0.6498	0.5647
g1 Statistic	-0.5506	-0.4953	-0.7136

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Data Partition	ND4 Outgroups	12S/16S Ancestor	12S/16S Outgroups
Number of Taxa	19	12	17
Total No. Sites in Analysis	753	667	668
No. Variable Sites (V)	344	112	159
No. informative Sites (I)	263	58	89
Ratio I/V	0.7645	0.5179	0.5597
%A (mean)	0.3501	0.3386	0.3419
%C (mean)	0.2602	0.2427	0.2423
%G (mean)	0.1122	0.1991	0.1974
%T (mean)	0.2776	0.2196	0.2184
No. Trees	1	4	7
Tree Length	1212	210	375
Consistency Index (CI)	0.4315	0.6333	0.5680
Retention Index (RI)	0.3667	0.4380	0.4510
Rescaled Consistency Index (RC)	0.1582	0.2774	0.2560
Homoplasy Index (HI)	0.5685	0.3667	0.4320
CI excluding uninformative	0.3787	0.4934	0.4527
HI excluding uninformative	0.6213	0.5066	0.5473
g1 Statistic	-0.5653	-0.5423	-0.6507

Data Partition	Combined mtDNA Ancestor	Combined mtDNA Outgroups	All Data Ancestor
Number of Taxa	14	19	14
Total No. Sites in Analysis	2451	2451	2483
No. Variable Sites (V)	826	976	858
No. informative Sites (I)	559	719	590
Ratio I/V	0.6768	0.7367	0.6876
%A (mean)	0.3234	0.3238	NA
%C (mean)	0.2577	0.2572	NA
%G (mean)	0.1390	0.1402	NA
%T (mean)	0.2800	0.2788	NA
No. Trees	1	1	2
Tree Length	2137	3413	2260
Consistency Index (CI)	0.5040	0.4230	0.5013
Retention Index (RI)	0.3461	0.3410	0.3523
Rescaled Consistency Index (RC)	0.1744	0.1440	0.1766
Homoplasy Index (HI)	0.4960	0.5770	0.4987
CI excluding uninformative	0.4204	0.3637	0.4215
HI excluding uninformative	0.5796	0.6363	0.5785
g1 Statistic	-0.7748	-0.6408	-0.7543

Cytochrome *b* had the highest ratio of informative to variable (I/V) characters, (0.705 – 0.773), but ND4 was similar (0.69 - 0.765). The I/V ratio for 12S/16S rRNA was much lower (0.518 – 0.56). The overall number and proportion of variable sites was much lower in the 12S/16S rRNA data set than either the cytochrome *b* or ND4 data sets.

The model of evolution chosen by Modeltest (TrN+I+Γ) for maximum likelihood analyses indicated all genetic data exhibited among site rate variation. Distance plots for cytochrome *b* and ND4 data sets (Figures 3-4) supported this assumptions. Transitions in third position codons exhibited the largest variation from other positions in both genes. Estimated rates of change for third positions were about an order of magnitude greater than second position rates (Table 2). First positions also showed a tendency towards high rates of change, but not to the same degree as third positions (only about 3-4 times the rate of second positions). Rates of change were slightly higher in cytochrome *b* than ND4.

All data sets (including weighted partitions) contained significant ($p < 0.01$) phylogenetic signal according to skewness statistics, indicating they were more structured than random noise and were appropriate for phylogenetic analysis. Permutation tail probability tests corroborated this result ($p = 0.01$). Results from partition homogeneity tests were mixed. These tests supported combining all the molecular data partitions together at the genetic level ($p = 0.44$) and at the codon level ($p = 0.54$) regardless of whether weights were applied. However, the tests did not support combining genes and morphology ($p < 0.01$). Genetic data were analyzed separately and jointly. Morphological data were

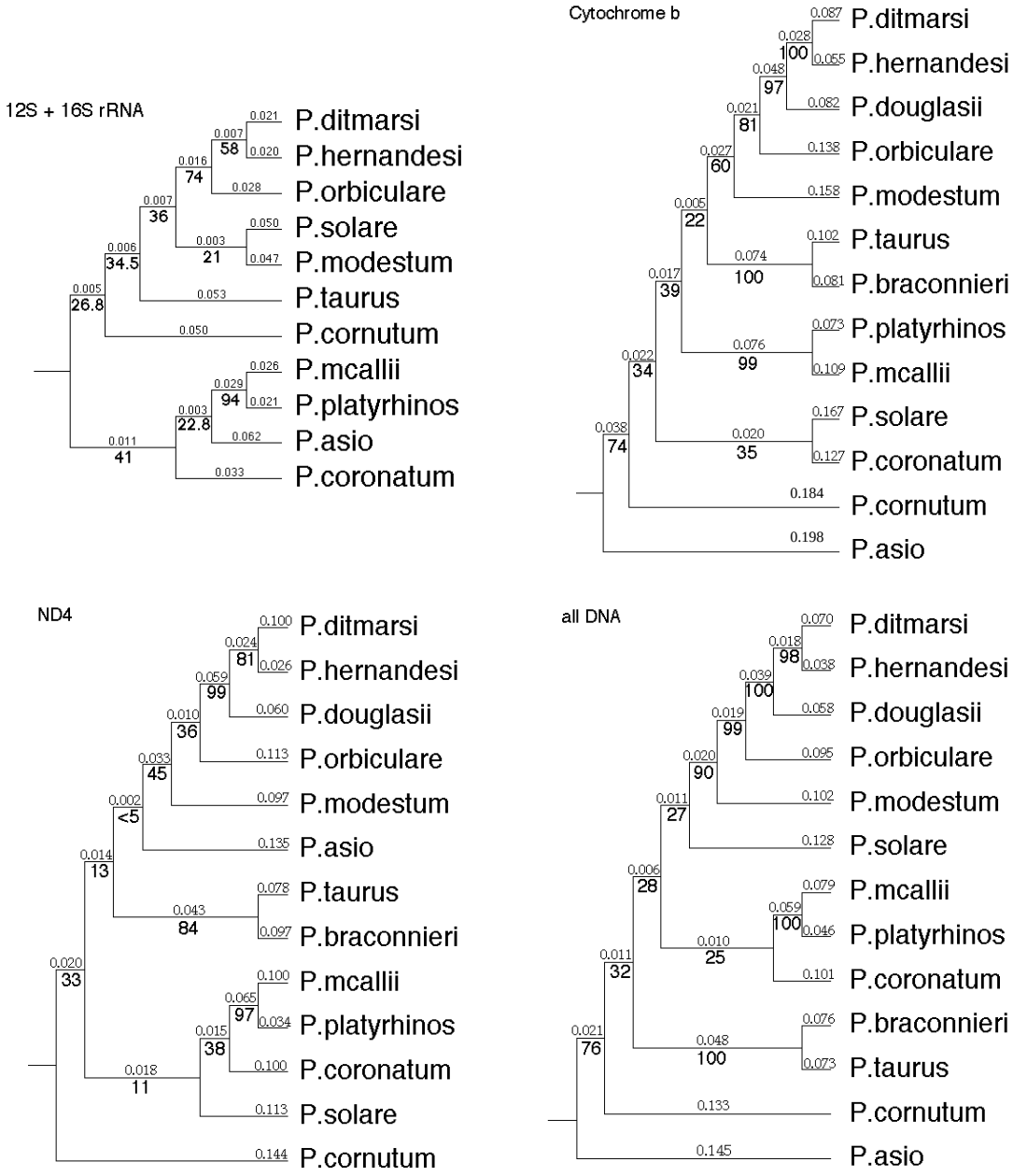
analyzed separately except for one analysis where it was combined with all molecular data to determine if any information from partitioned Bremer values could shed any light on the nature of conflict between data sets.

Parsimony and Maximum Likelihood Analyses

Topologies representing best estimates for phylogeny using maximum likelihood (ML) optimization of each mitochondrial data set independently and a combined analysis are shown in Figure 5. In all analyses, several relationships were present: P. mcallii and P. platyrhinos are well supported as sister taxa, as well as P. taurus and P. braconnieri (except in the 12S/16S analyses since P. braconnieri had not been sequenced for these genes). Though not strongly supported in all ML analyses, the "short-horned lizards," P. ditmarsi, P. hernandesi, P. douglasii and P. orbiculare, formed a monophyletic group, usually with P. modestum as a sister taxon (except in the 12S and 16S rRNA analysis where the species was joined with P. solare as the sister group to the short-horned lizards). In three of four cases, P. cornutum came out as a basal taxon. Remaining taxa (P. coronatum, P. asio and P. solare) moved around in the topologies of each analysis and showed no consistent affinities for placement. The combined DNA analysis had seven well-supported clades all but one of which were found in analyses of individual genes. The outlying clade was a branch representing all species except P. asio and P. cornutum, which were placed outside and basal to the rest of the genus.

Figure 5: Maximum Likelihood Topologies for Mitochondrial Genes.

Topologies resulting from maximum likelihood analysis of each mitochondrial data set and the mitochondrial genes combined. Numbers on top of branches represent branch lengths, number below branches represent bootstrap support values.



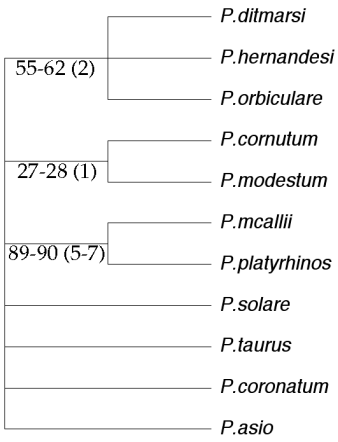
Topologies resolved in parsimony and weighted parsimony analyses of the mitochondrial genes were very similar to maximum likelihood topologies (Figures 6 and 7). The short-horned lizard group (P. ditmarsi, P. hernandesi, P. douglasii and P. orbiculare) appeared monophyletic in all but the ND4 analyses where P. modestum appeared within the group (making it paraphyletic). Differential weighting had no effect on this result. Phrynosoma modestum was often associated with the short-horned group either as a sister taxon or paired with another taxon as part of a sister group. In all analyses, P. platyrhinos and P. mcallii were sister taxa as well as P. taurus and P. braconnieri (except in the 12S/16S rRNA analysis that omitted P. braconnieri). Phrynosoma cornutum appeared as a basal taxon in most analyses, and the remaining taxa appeared in different places on different topologies. Maximum likelihood and parsimony analyses resolved the same well and moderately supported clades. The primary difference between weighted parsimony analyses and unweighted parsimony and ML analyses was slightly reduced levels of bootstrap support given for otherwise well supported clades. This affected the codon-weighted topologies only; transition/transversion weighted analyses showed virtually no difference. From a qualitative perspective, method of phylogenetic reconstruction appeared not to affect well-supported clades for a given data set. Results from different data sets did resolve different topologies overall, but well supported clades repeatedly appeared in analyses of different data sets.

Figure 6: Topologies for All Data Partitions Using Unweighted Parsimony.

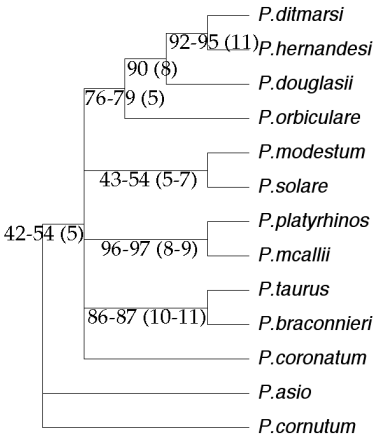
Topologies resulting from unweighted parsimony analyses of each data partition. In most cases, more than one best tree was found and a consensus of those topologies is shown rather than each tree. Bootstrap support is shown below each branch with decay index (Bremer support) in parentheses. Bootstrap values and Bremer indices were assumed to confer similar levels of support for a given clade, assuming bootstrap values of 70% or greater represented accurate clades with respect to the true tree (Hillis and Bull, 1993), and Bremer values greater than 5 reflected strong evidence of branch stability (Frost et al., 2001; Pellegrino et al., 2001; Flores-Villela et al., 2000). See Table 4 for information on number of trees found for each partition.

next page

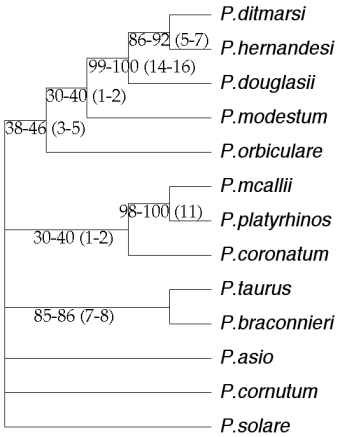
12S + 16S rRNA



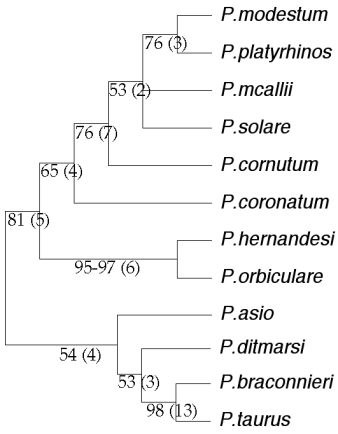
Cytochrome *b*



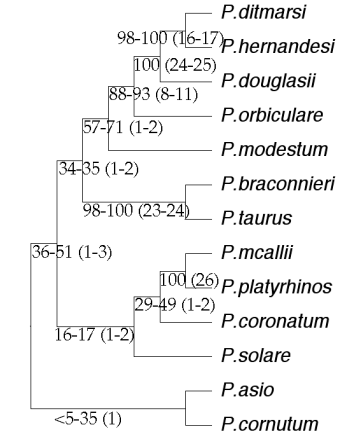
ND4



Morphology



All DNA



All Data

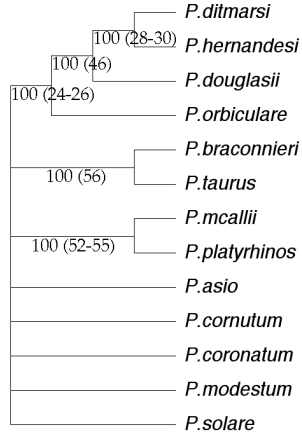
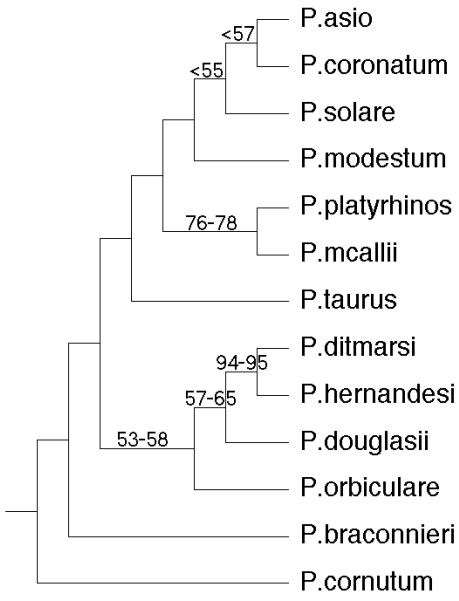


Figure 7: Consensus Trees for Codon-weighted and Transition/Transversion Weighted Parsimony.

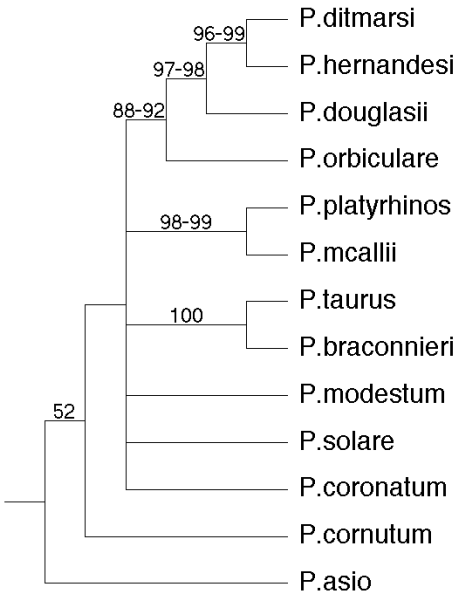
Top graphs show the consensus of all cytochrome *b* analyses and lower graphs show ND4 consensus using weighting schemes representing different rates of change for each codon position and transition and transversion rates. All strongly supported relationships found are identical to prior results from unweighted parsimony and maximum likelihood analyses. Codon weighting generally reduced bootstrap support given to specific clades, but transition/transversion (ti/tv) weighting generally had no effect. Bootstrap support shown on ti/tv consensus trees was calculated on original fundamental trees and falls within the values from unweighted parsimony and maximum likelihood analyses.

next page

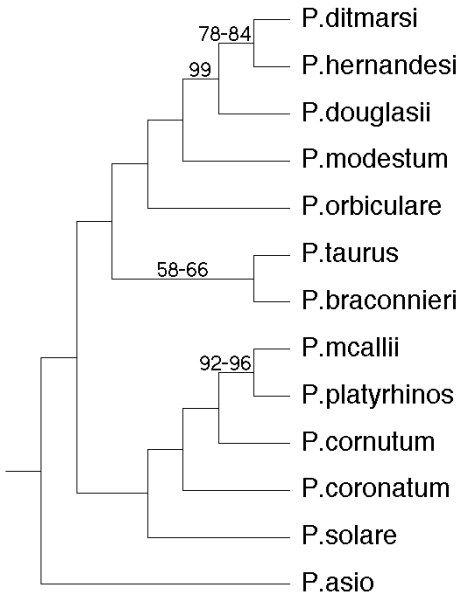
Cytochrome b: Codon Position Weighting



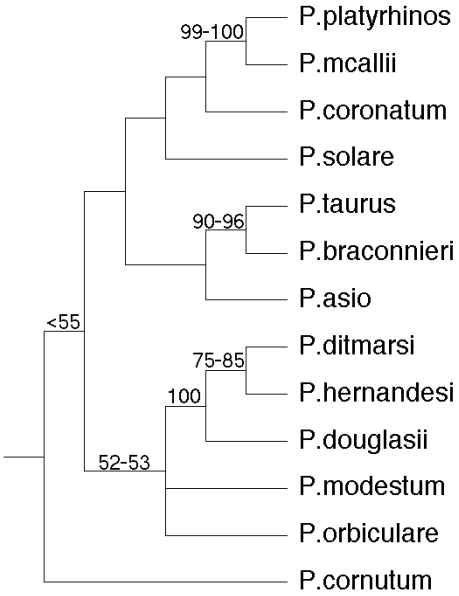
Cytochrome b: Ti/Tv Weighting



ND4: Codon Position Weighting



ND4: Ti/Tv Weighting



The 12S/16S rRNA data sets resolved the least number of well-supported relationships in both ML and parsimony analyses. Only the P. mcallii – P. platyrhinos clade was well supported by this data set. This data set did not include all taxa, and had the fewest number of characters that contained the fewest number of informative sites and lowest ratio of informative to variable sites (Table 4). One additional moderately supported clade included three of the short-horned lizard species, but relationships with the group were not well resolved.

Cytochrome *b* and ND4 showed similar ratios of informative to variable characters, and both data sets strongly supported four identical taxonomic relationships in both ML and parsimony analyses. The relationships strongly supported were sister pairing of P. ditmarsi with P. hernandesi and their sister relationship to P. douglasii, P. mcallii and P. platyrhinos pairing, and the pairing of P. braconnieri and P. taurus. One relationship moderately supported in both analyses included P. orbiculare with the rest of the short-horned lizard species; however, in ND4, P. modestum was also included in the group. Both data sets weakly supported P. cornutum as a basal taxon to other Phrynosoma.

Maximum likelihood and parsimony methods recovered the same five strongly supported relationships in combined mitochondrial DNA analyses. The five clades included grouping the short-horned lizards as a monophyletic group (P. ditmarsi, P. hernandesi, P. douglasii and P. orbiculare) and the sister relationships of P. braconnieri - P. taurus and P. mcallii - P. platyrhinos. Phrynosoma asio and P. cornutum were weakly to moderately supported as basal taxa to the rest of the genus. As expected with increasing amounts of data,

phylogenetic relationships were consistent and showed increasing support and stability in the combined molecular analyses. Each strongly supported clade in separate analyses received higher levels of support in the combined genetic analysis. However, topologies produced in analyses with the morphological data were quite different from the topologies produced with genetic data.

Only one clade (P. taurus and P. braconnieri) strongly supported in the morphological analysis was also strongly supported by the genetic data (Figure 6). The only other strongly supported clade in the morphological analysis was P. hernandesi and P. orbiculare. In genetic analyses, these two taxa formed part of a monophyletic group with P. ditmarsi and P. douglasii, but morphological analyses weakly supported P. ditmarsi as a sister taxon to P. taurus and P. braconnieri instead of placing P. ditmarsi with other short-horned lizards (as defined in this study). Morphological analyses moderately supported three other clades that were not found in any of the genetic data sets. Unlike the genetic analyses, neither P. asio nor P. cornutum appeared as basal taxa to the remaining genus. A strict consensus tree of morphological and molecular data only retained a single resolved clade (P. braconnieri and P. taurus) and preserved no other structure in the rest of the tree. The two data types (genes and morphology) resolved very different relationships overall, and corroborated the partition homogeneity test results that showed these two data sets may not be combinable.

Taxonomic Congruence

Strict consensus trees from parsimony and maximum likelihood analyses for each molecular data set were made to summarize agreement among topologies

from different phylogenetic methods (Figure 8). Consensus trees reiterated the well-supported clades in each analysis. Though certain elements were consistent between different topologies, incongruence was still present. Results from Wilcoxon signed-rank (Templeton) and winning-sites tests suggested more incongruence between and within data sets than using parametric bootstrap (Monte Carlo) simulations.

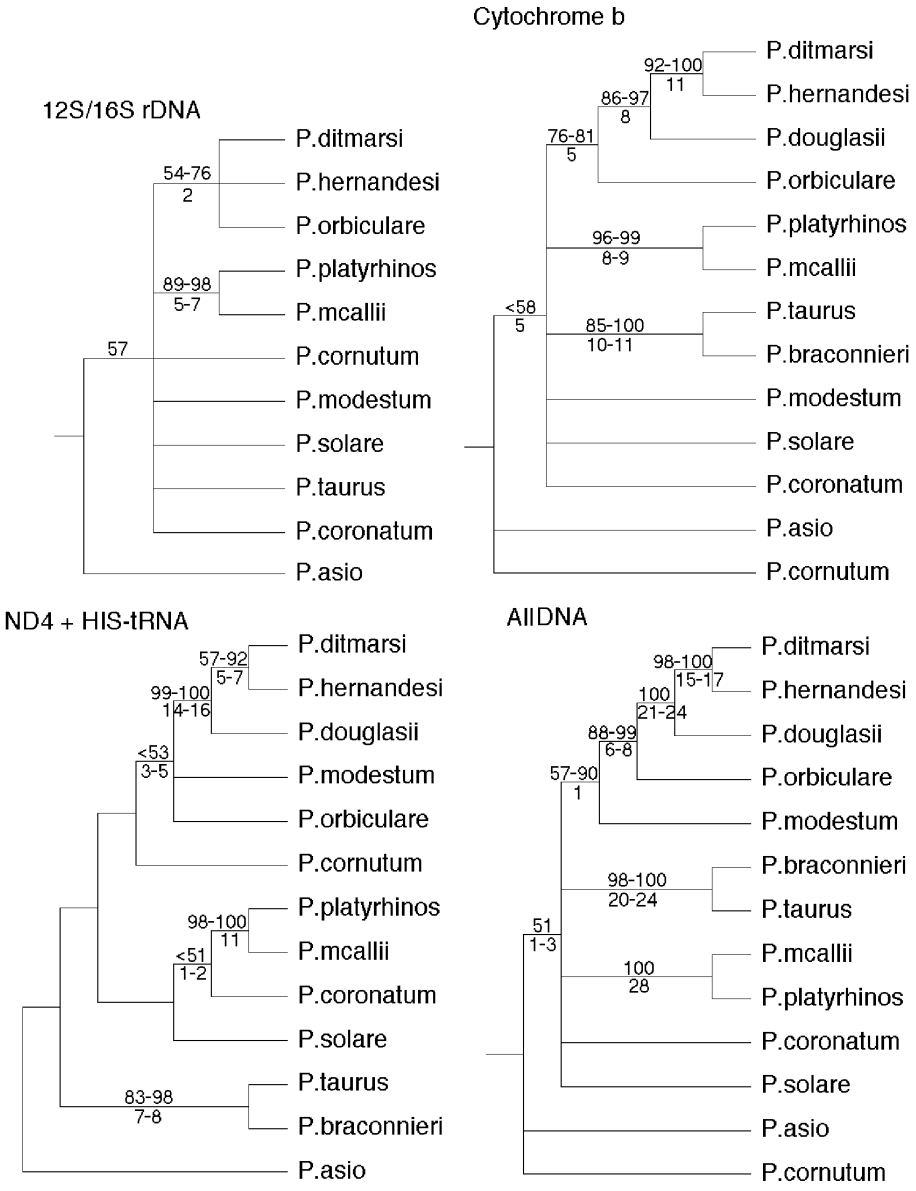
Using Wilcoxon signed-rank and winning-sites tests, phylogenetic trees from parsimony and maximum likelihood analyses within each genetic data partition were different but not incongruent ($p > 0.11$). Using either a computed ancestor or the individual outgroup taxa also had no apparent influence on ingroup topology within a data partition ($p > 0.12$, Templeton and winning-sites tests). Combining all molecular data together under different weighting schemes produced different topologies; however, incongruence was not observed between the different topologies ($p > 0.5838$, Templeton; $p > 0.4594$, winning-sites).

Although intragenic incongruence was not seen in the Wilcoxon signed-rank and winning-sites tests, intergenic incongruence was present. Optimal trees from different analyses were used as constraints on opposing data sets and those constrained trees were significantly rejected in most cases. Cytochrome *b* topologies from parsimony analyses constrained on the ND4 data sets had significantly larger tree scores than ND4 trees ($p < 0.02$, Templeton; $p < 0.05$, winning-sites), but cytochrome *b* trees from ML analyses did not ($p > 0.06$, Templeton; $p > 0.11$, winning-sites). All ML and parsimony topologies from

Figure 8: Consensus Trees of all Analyses for Each Data Partition.

Strict consensus trees of parsimony and maximum likelihood analyses for each data partition and combined analysis of genetic data are shown. Numbers above a branch represent the range of bootstrap values obtained from separate analyses and numbers below a clade are Bremer support indices. Bootstrap values and Bremer indices were assumed to confer similar levels of support for a given clade, assuming bootstrap values of 70% or greater represented accurate clades with respect to the true tree (Hillis and Bull, 1993), and Bremer values greater than 5 reflected strong evidence of branch stability (Frost et al., 2001; Pellegrino et al., 2001; Flores-Villela et al., 2000).

next page



ND4 analyses tested on the cytochrome *b* data were rejected over the cytochrome *b* topology ($p < 0.01$, Templeton and winning-sites). Applying any morphological or 12S/16S rRNA topology as a constraint to either ND4 or cytochrome *b* data set always produced trees that were rejected in favor of the tree from the original data

set ($p < 0.001$, Templeton and winning-sites). These results suggested in most cases, topologies within data sets were congruent independent of method of phylogenetic analysis, but incongruence was present between data sets.

Parametric bootstrap simulations also suggested taxonomic incongruence was present but not to the same extent as the Templeton and winning-sites tests (Table 5). Topologies from parsimony and maximum likelihood analyses of the 12S/16S rRNA data were strongly rejected by ND4 and cytochrome *b* ($p < 0.02$). Topologies from analyses of the morphological data also were rejected by both ND4 and cytochrome *b* ($p < 0.01$). Results comparing ND4 and cytochrome *b*, however, were less straightforward. Imposing cytochrome *b* topologies from ML or parsimony analyses on the ND4 data set did not result in significant differences in tree scores ($p > 0.07$). The reverse was not true; imposing the constraint of topologies produced in parsimony analyses of ND4 on the cytochrome *b* data resulted in rejection of all the ND4 topologies ($p \leq 0.01$). Cytochrome *b* and ND4 data sets also were different in how they responded to tests with different phylogenetic methods.

Topologies from ML analyses of ND4 could not be rejected in favor of the parsimony topology ($p = 0.55$) from the same data set. Neither ML topologies nor parsimony topologies from the combined DNA analyses could be rejected by the ND4 data set ($p > 0.07$). A topology favoring monophyly of viviparous species was rejected ($p < 0.01$) and so was a topology from a parsimony analysis of all the data combined ($p < 0.01$) using the ND4 data. The topology from ML analyses of cytochrome *b* was rejected in favor of parsimony topologies for the

Table 5: Parametric Bootstrap Simulations and Tests of Alternative Hypotheses.

Parametric bootstrap simulations were used to test alternative hypotheses for topologies produced with different data sets and optimizations. One additional test was done to determine whether viviparous species formed a monophyletic group. "*" indicates significant differences or incongruence between the topology and data set.

		Tree	Test		
Data set Used	Topology test	Score	Statistic	p-value	significant
ND4	Parsimony tree from ND4	744			
.-P.brac	(ND4 adjusted for Morph)+	706			
.-Pbrac+Pdoug	(ND4 adjusted for 12s/16s)~+C5	654			
	ML tree from cytb	757	13	0.35	
	Parsimony tree from cytb	764	20	0.07	
	ML tree from ND4	748	4	0.55	
	ML tree from 12s/16s ~	677	23	0.02	*
	Parsimony tree from 12s/16s	812	68	0.01	*
	ML tree from all DNA data	756	12	0.22	
	Parsimony tree from all DNA data	750	6	0.48	
	Parsimony tree from morphology-1 +	770	64	<0.01	*
	Parsimony tree from morphology-2 +	769	63	<0.01	*
	Parsimony tree from all data	764	20	<0.01	*
	Viviparous species are monophyletic	756	12	<0.01	*
		Tree	Test		
Data set Used	Topology test	Score	Statistic	p-value	significant
Cytb	Parsimony tree from cytb	1167			
	ML tree from cytb	1180	13	<0.01	*
	ML tree from ND4	1191	24	0.01	*
	Parsimony tree from ND4	1205	38	<0.01	*
	ML tree from 12s/16s	1220	53	<0.01	*
	Parsimony tree from 12s/16s	1266	99	<0.01	*
	ML tree from all DNA data	1178	11	0.13	
	Parsimony tree from all DNA data	1174	7	0.56	
	Parsimony tree from morphology - 1	1293	126	<0.01	*
	Parsimony tree from morphology - 2	1289	122	<0.01	*
	Parsimony tree from all data	1170	3	0.59	
	Viviparous species are monophyletic	1175	8	0.12	
		Tree	Test		
Data set Used	Topology test	Score	Statistic	p-value	significant
allDNA	ML tree from cytb	2153	16	0.01	*
	Parsimony tree from cytb	2142	5	0.5	
	ML tree from ND4	2156	19	0.07	
	Parsimony tree from ND4	2167	30	0.09	
	ML tree from 12s/16s	2242	105	<0.01	*
	Parsimony tree from 12s/16s	2292	155	<0.01	*
	ML tree from all DNA data	2145	8	0.11	
	Parsimony tree from morphology-1	2372	235	<0.01	*
	Parsimony tree from all data	2145	8	0.08	
	Viviparous species are monophyletic	2152	15	0.01	*

cytochrome *b* data set (suggesting an incongruence between phylogenetic methods). A topology favoring monophyly of viviparous species could not be rejected by the cytochrome *b* data as well as topologies from parsimony analysis of all the data combined and topologies from ML or parsimony analyses of all the molecular data combined. Cytochrome *b* and ND4 showed almost reverse patterns in which they rejected alternative hypotheses.

When all molecular data were combined, the ML topology from cytochrome *b*, ML and parsimony topologies from 12S/16S rRNA, the morphological and monophyly of viviparous taxa topologies were all rejected in favor of the combined molecular data topology created from parsimony analysis. Topologies from ML analysis of the combined data, ML and parsimony analyses of ND4 data, parsimony analysis of cytochrome *b* and all data could not be rejected.

Unlike results from Templeton and winning-sites tests, the results of parametric bootstrap simulations provided some indication that different molecular data sets may be congruent, but these results were ambiguous. Previous tests for data combinability (partition homogeneity tests) supported combining the molecular data. Topologies from individual analyses of each molecular data suggested that regions of well-supported clades existed and were consistent among the different data sets. However, tests of taxonomic congruency were unable to fully support the combinability of all the molecular data, especially 12S/16S rRNA with protein coding genes. Combining the morphological data with the molecular data was not supported by any test.

Partitioned Bremer support indices were added to the consensus tree of all phylogenetic analyses conducted on the combined mitochondrial data (Figure 9). A negative value indicates the data partition did not support a clade. The partitioned Bremer values showed that the data set conflicting most often with the consensus topology was 16S rRNA. Bremer values of "0" were common for the 12S rRNA indicating this data partition did not support or conflict with the topology shown. Bremer support indices did fail to show support by the ND4 data set for the P. ditmarsi – P. hernandesi clade, which the data set did support when analyzed independently. Cytochrome *b* (and 16S rRNA) data conflicted with the placement of P. modestum in the short-horned lizards.

Bremer support values were also calculated for a tree derived from the analysis of all data combined (Figure 10). This analysis showed that only a single clade, P. mcallii – P. platyrhinos, is positively supported by all data partitions and has high bootstrap support (Table 6). One other clade, P. taurus – P. braconnieri was supported by all but the 12S/16S rRNA data set (P. braconnieri was not sequenced for 12S/16S rRNA) and also received high bootstrap support. Though parsimony analysis identified this topology as one of the shortest trees, Bremer support suggested three of the clades were not supported by any data. In the other shortest tree (not shown), Bremer indices showed two clades from the parsimony analysis were not supported by any data, but the same well supported clades (both in terms of bootstrap and total Bremer support) were present, and the only clade receiving positive support from all data was again the P. mcallii – P. platyrhinos

Figure 9: Partitioned Bremer Support Indices on Combined mtDNA Tree.

Partitioned Bremer Support Indices calculated on combined mitochondrial consensus tree are shown to the left. Clades are labeled with letters above branches and total Bremer support values are below branches on the diagram. Gene order is the same for all branches as listed for clade A.

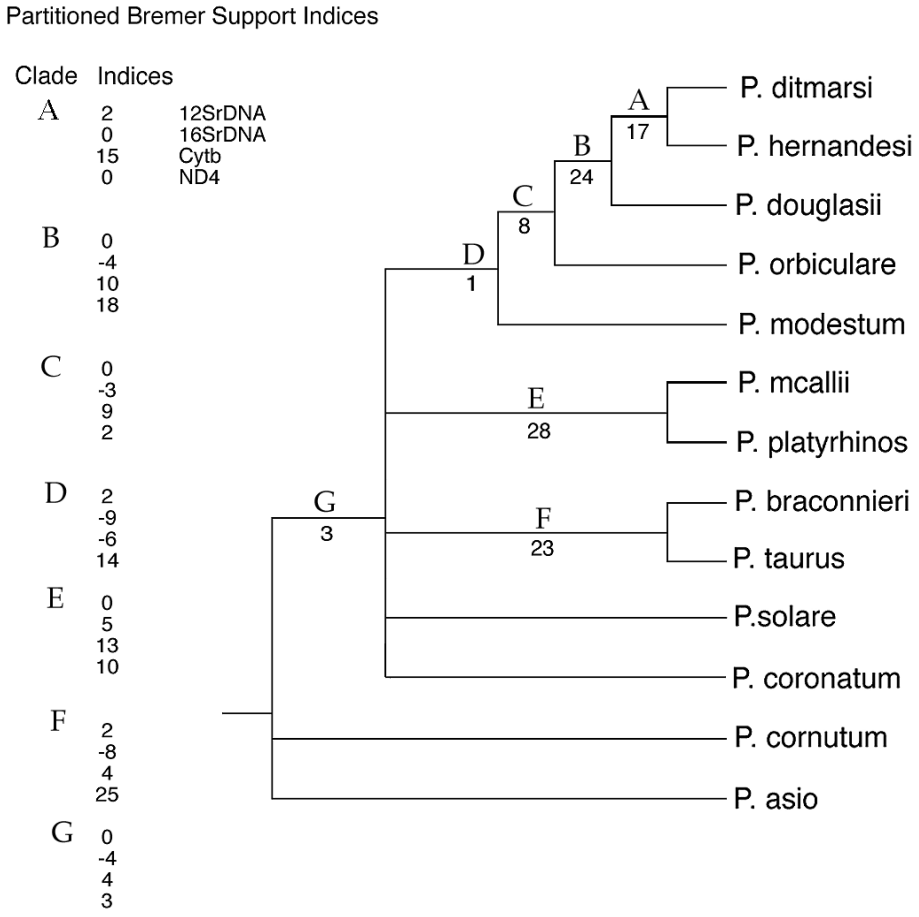


Figure 10: Combined Data Analysis.

One of two best trees found in parsimony analyses using both branch-and-bound and heuristic searches using all available molecular and morphological data. Branch lengths are shown above branches, and clade identification is shown below branches. Corresponding bootstrap (BT), partitioned Bremer support (PBS), and total Bremer support values are listed in Table 6.

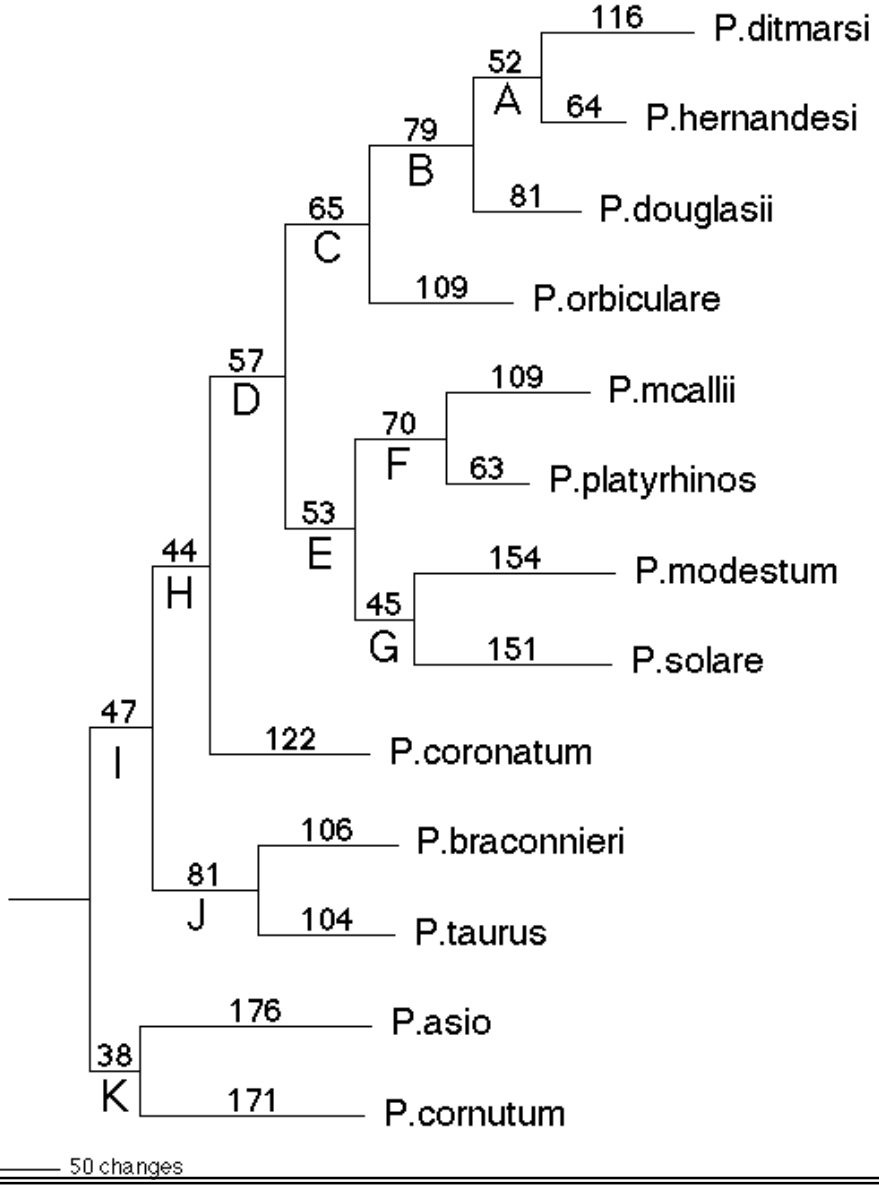


Table 6: Partitioned Bremer Support Indices for Combined Data Analysis

All data were combined in a "total evidence" analysis even though tests suggested that morphological and molecular data were incongruent and should not be combined. Bootstrap and Bremer support indices are shown for the topology in Figure 10. Bremer support indices are broken into partitions represented by each data set. All data partitions are listed in the same order: 12S rRNA, 16S rRNA, cytochrome *b*, ND4, morphology.

CLADE	BT	PBS	Total Bremer	CLADE	BT	PBS	Total Bremer
A	100	0 0 20 8 0	28	**G**	32	-6 8 13 -23 14	6
B	100	-2 6 18 24 0	46	**H**	21	0 0 0 0 0	0
C	100	-3 6 19 -17 23	28	**I**	37	-6 6 19 -21 12	10
D	28	0 0 0 0 0	0	**J**	100	0 0 20 14 18	52
E	22	-4 0 -4 -4 16	4	**K**	<2	0 0 0 0 0	0
F	100	2 7 13 28 2	52				

clade. Ironically, the morphological data showed only positive (rather than negative) support for clades, and in five cases the data did not support a clade. Data sets showing most conflict were the 12S and ND4 data sets. The 12S data conflicted with five clades and ND4 conflicted with four clades, but the ND4 conflicts appeared to be stronger assuming larger negative values represent stronger conflict. Three of the four clades showing the strongest conflict were related to exclusion or inclusion of P. modestum relative to where the data supported this taxon's placement in independent analyses of ND4 data. Partitioned Bremer indices in the combined analysis were similar to the results from taxonomic congruency tests and analysis of separate data sets in that they supported well-resolved branches found in most of the other analyses and revealed points of conflict.

An additional analysis was conducted by selectively removing taxa from phylogenetic estimates and recalculating the skewness (g_1) statistic. Previous analyses showed all data contained significant signal, but when members of pairs of strongly supported clades (from the molecular analyses) were removed, skewness statistics suggested the data were no longer valid for phylogenetic analyses. Removal of P. platyrhinos or P. mcallii in combination with P. douglasii and P. hernandesi and either one of the P. taurus - P. braconnieri clade was sufficient to reduce the signal to the level of random noise. Alternatively, removing any three of the short-horned lizard group with one member of both pairs consisting of P. platyrhinos - P. mcallii and P. braconnieri – P. taurus also reduced the phylogenetic signal to random noise. Removing four or five of the

stated taxa was necessary to produce this result while removing taxa outside of these clades had no effect on the g_1 statistic, indicating most signal from molecular data was contained in these taxa.

DISCUSSION

Analyses of multiple data sets can be problematic if data are incongruent (Bull et al., 1993; Miyamoto and Fitch, 1995). Initial tests for congruency between data sets using the partition homogeneity test suggested that all molecular data were combinable, but morphological data were not combinable with the molecular data. Independent analyses of each data set and a combined molecular data set initially supported this result in a qualitative manner; well-supported clades in each molecular partition were present across multiple analyses and results of morphological analyses supported different topologies compared to the molecular data. Combined molecular data analyses appeared to confirm that well supported clades from independent analyses were supported by combining the molecular data. However, using quantitative tests for taxonomic congruence between data sets revealed conflict did exist between them in contrast to the partition homogeneity test and qualitative assessments of congruence. Dowton and Austin (2002) suggested the partition homogeneity test may not be an effective measure of congruence when data sets differ in size. In this study, cytochrome *b* contained 1.5 – 5.2 times the number of informative sites compared to ND4 and 12S/16S rRNA. The congruence observed between partitions may be an artifact of the larger contribution of the cytochrome *b* data to the shortest tree

rather than a true measure of congruence between data sets, leaving open the possibility these data may not fit the combinability test after all.

Templeton and winning-sites tests suggested that conflict was present between all data sets – including the molecular data. However, parametric bootstrap simulations suggested that at least the ND4 and cytochrome *b* data might be congruent, though these results were still ambiguous. A conservative approach in light of this ambiguity would be to keep all data separate. Morphology and 12S/16S rRNA data sets were still most in conflict with the other data sets. Partitioned Bremer support indices indicated in combined analyses of all the molecular data that most of the conflict appeared to be with the 12S/16S rRNA data. Many studies analyzing multiple data sets often combine all mitochondrial genetic data as a single partition arguing partition homogeneity tests or a qualitative assessment of congruence (e.g. looking for compatible nodes with strong support) verify they are combinable and all mitochondrial data are linked and therefore not independent. If these genes do in fact show incongruence using alternative methods, these assumptions could be reevaluated; even though linked on the mitochondrial genome, these genes may still respond to different selective pressures and exhibit different evolutionary histories.

Partitioned Bremer support indices were imposed on a combined tree of all data and revealed that while several clades were well supported by bootstrap and total Bremer support indices, several nodes resolved in parsimony analyses were supported by none of the data sets. These unsupported nodes also receive very low bootstrap support and were not nodes supported in any other analyses.

Partitioned Bremer indices also suggested in the combined analyses with all data that morphological data did not always conflict with the molecular data, but often simply did not support clades that were found by parsimony analysis. In fact, in a combined analysis, the morphological data showed positive support for clades not supported in the independent analysis of morphological data alone, a reversal in position if only topologies from separate analyses are compared for taxonomic congruence.

Independent analyses of each data partition do support several relationships also supported in combined analyses: a clade representing up to four species of montane, viviparous, short-horned lizards (P. douglasii, P. orbiculare, P. ditmarsi, P. hernandesi), a separate clade joining the two remaining viviparous species (P. taurus and P. braconnieri) and one clade containing two oviparous species (P. mcallii and P. platyrhinos). No novel, well-supported branches were resolved by combined analyses. The majority of comparisons using parametric bootstrapping simulations significantly rejected the monophyly of all viviparous species. Monophyly is also rejected in combined analyses and all consensus trees of each independent data set; monophyly of viviparous species is highly questionable.

A final set of analyses using the skewness statistic showed that if taxa belonging to those clades that were most resolved and had the highest support were removed from analyses that the data were no longer appropriate for phylogenetic analyses. In other words, the data contained sufficient signal to resolve only a subset of the relationships in the genus. These results could mislead

taxonomic congruence anayses and suggest the data conflicted when in fact the data lacked sufficient signal. Poor resolution and support in parts of the tree can be attributed to lack of sufficient signal. This underlying problem can be seen in the molecular data.

The12S/16S rRNA data did not vary enough to provide much signal throughout the tree. These data provided little phylogenetic information to resolve many relationships with only 58 informative sites available for analyzing 12 taxa and the highest proportion of invariable sites. A low proportion of variable sites can lend low phylogenetic signal to an analysis (Yang, 1998), and a small number of nucleotides may also give incorrect phylogenies (Nei, et al., 1998). These characteristics may have been responsible for the data strongly supporting only a single, recently diverged clade, conflicts with the other molecular data, and providing negative support for well-supported relationships in combined analyses. Also, the lack of data for two taxa (P. braconnieri and P. douglasii) may have impacted the ability to resolve clades or could give misleading results.

Cytochrome *b* and ND4 data contained more informative characters and were more variable. Variation was highest in third position codons where substitution rates were an order of magnitude higher than second position codons. If rates dominating cytochrome *b* and ND4 data are too high, they can limit resolving power in the phylogeny (Yang, 1995). Nucleotide changes contribute significant noise to the data if the number of changes between nodes is sufficiently high enough to randomize character states (Hillis and Huelsenbeck,

1992). Randomization was noted by the removal of taxa (e.g. P. platyrhinos, P. orbiculare, and P. taurus) that lead to skewness values indicating loss of phylogenetic structure in the data. Cytochrome *b* is known to exhibit problems in resolving deeper relationships in other taxa (Meyer and Wilson, 1990; Graybeal, 1993; Halanych and Robinson, 1999). Resolved clades were found at the tips of the phylogeny among closely related species, while unresolved portions of the phylogenies were at basal positions. ND4 appeared to follow the same pattern in this study and did not resolve basal relationships with strong support.

Basal relationships represent older phylogenetic events than tip relationships and early divergences may be obscured by considering only extant taxa. Fossils representing Phrynosoma are found sporadically throughout the paleontological record. Most have been found mostly from the Pliocene and Pleistocene (e.g. Brattstrom, 1955**;** Etheridge, 1958; Rickart, 1976; Schultze, et al., 1985). The oldest known Phrynosoma fossil of a nearly complete right maxilla is currently assigned to P. douglasii (Robinson and Van Devender, 1973). Species designation of the fossil was made on the basis of its close similarity in maxillary morphology, tooth space count, and ecological distributions of seven extant Phrynosoma found in the United States and dates to mid-Miocene, 17-20 million years ago. This age suggests the clade containing the short-horned lizards (P. orbiculare, P. douglasii, P. hernandesi, and P. ditmarsi) is at least 17 million years old and probably arose earlier since the fossil represents only one member of the clade. While cytochrome *b* sequences have been used to resolve relationships both older and younger than this, their utility in resolving

Phrynosoma basal relationships older than 17 million years appears limited (Meyer and Wilson, 1990; Cantatore, et al., 1994; Trépanier and Murphy, 2001). Unresolved portions of the Phrynosoma phylogeny appear to represent more ancient divergences that will require different genes with more appropriate substitution rates or morphological traits to discriminate these older taxonomic relationships (Russo, 1997). Inclusion of more fossils may bridge morphological gaps and return more characters to the analyses.

One potential problem in the current morphological data is that P. douglasii and P. hernandesi are combined into a single taxon, P. hernandesi. This has unknown effects on the phylogenetic structure of the data, since molecular data indicated P. ditmarsi splits the relationship of these two species. In morphological analyses, P. ditmarsi is not included in the P. hernandesi - P. orbiculare clade, but P. ditmarsi falls as sister taxon to P. taurus and P. braconnieri. In all molecular analyses, P. taurus and P. braconnieri form a unique and well supported, independent clade distant from P. ditmarsi. Conflict between molecular and morphological data sets could arise due to the presence of convergence in morphological data, different evolutionary rates between characters, introgressive hybridization, lineage sorting, or nonindependence of characters (de Queiroz, et al, 1995; Joy and Conn, 2001, Sota and Vogler, 2001). In the morphological analysis, the placement of P. ditmarsi with P. braconnieri and P. taurus is supported by only two changes, one that is unique (reduction in number of caudal vertebrae). Despite taxonomic and character based conflicts, partitioned Bremer support in the combined analyses showed morphological

characters were not in conflict with all the molecular data except in one clade. When the morphological traits are mapped on the mitochondrial DNA tree using MacClade (not shown), the clade containing the four species of short-horned lizards are supported by seven changes, three that are unique. All changes are features of the skull and region surrounding the maxilla and mandible. Combined data analysis showed that despite lower numbers of informative characters and apparent incongruence with molecular data in separate analyses, morphological data contributed significant positive support in well-supported clades. These results also support growing evidence that morphological data, though often outnumbered, are not swamped by molecular data sets and differentially weighting characters is unnecessary to accommodate differences in number of informative characters between data sets (Baker et al. 1998; Frost, et al., 2001).

Strongly supported relationships in unweighted, combined analyses were present in separate analyses and analyses using different weighting criteria. Weakly supported groups in separate analyses were not improved by any alternative weighting schemes or combining data together. Clades with weak support appear to represent areas of uncertainty in the phylogeny or areas where data become randomized. Conflicts between trees produced from different data sets were present where no data set resolved relationships. These results supported including all data in analyses without differential removal or weighting characters and leaving areas in conflict as areas of phylogenetic uncertainty to be resolved by additional data (Wiens, 1998; Yang, 1998; Baker et al. 2001). The trees from unweighted parsimony analyses of all combined data were chosen to represent the

best current phylogenetic hypotheses for Phrynosoma with the understanding that basal relationships remain unsupported by currently available data. These trees are chosen because the relationships resolved in these analyses by well-supported branches are present in the individual analyses except for the morphological data. When all data are combined, the morphological data actually do show positive support for three out of five clades that receive high bootstrap support. The two well-supported clades remaining are simply not supported by the morphological data and these clades include P. douglasii and P. hernandesi for which the morphological data cannot discriminate between.

The benefit of multiple analyses on multiple data sets, using data as independent partitions and combined together was shown in this study. If a dogmatic approach had been used to limit analyses only to total evidence or consensus analyses, information about the nature of conflicts and ultimately the nature of the data would have been overlooked. By fully exploring the data and using all available analytical techniques, it became evident that the data themselves were limiting in their power to resolve basal relationships in Phrynosoma. Additional data are needed to tease apart these basal relationships. The data will need to contain higher levels of informative and variable characters than the 12S/16S rRNA data, but must also exhibit nucleotide substitution rates lower than cytochrome *b* and ND4 data to avoid randomization of the data. Some of these data sources could be found in additional molecular sequences (perhaps nuclear genes), but other potential data sources are the inclusion of more fossil data and additional morphological characters (see Chapter 4).

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Chapter Two: Comparative Reproductive Ecology of Mexican Horned Lizards and Evolution of Viviparity

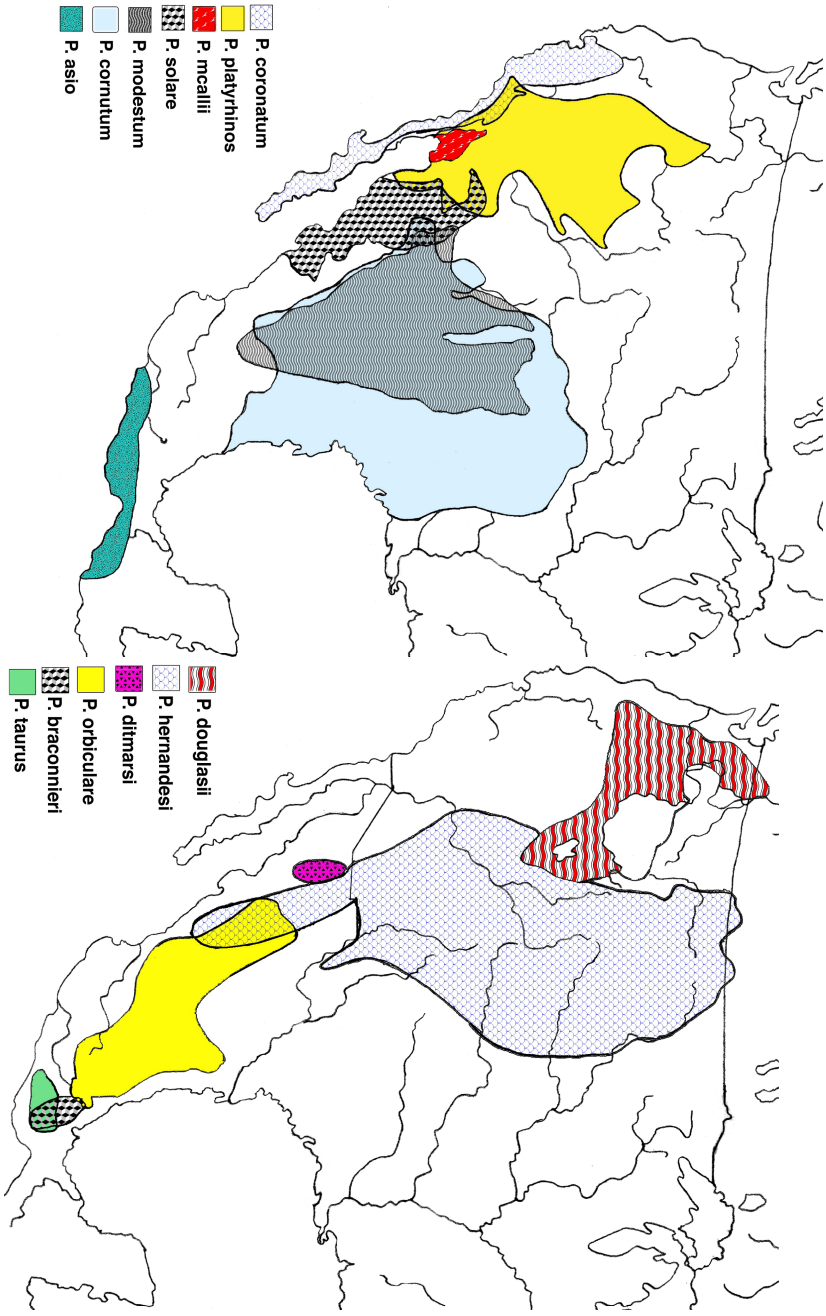
Phrynosoma (horned lizards) exhibit a variety of reproductive tactics including both early and late maturing species and species with small to large clutch sizes (Howard, 1974). Both oviparous (egg laying) and viviparous (live bearing) modes of reproduction are represented in the genus (Montanucci, 1989; Zamudio and Parra-Olea, 2000). Viviparous species occur throughout North America, from southern Canada through south-central Mexico (Figure 11). Oviparous species occur from southern Idaho to northern Guatemala.

Very little information is known about the general ecology or reproductive characteristics of Mexican Phrynosoma. Few museum specimens exist for most species, and over seventy-five percent of them have been damaged by extensive use and abuse, including complete removal and loss of internal organs (Montanucci, 1994; personal observation). Few field studies have collected even basic information on reproduction, including seasonal and daily activity periods, mating season, ovarian or testicular cycles, clutch or brood size. The following research was conducted in Mexico to collect reproductive information and basic ecological data on this poorly known group of horned lizards. In addition to field collections and observations, new material from Mexican museums augmented the existing published record. Some reproductive and ecological data were used to study the evolution of viviparity in the genus.

The influence of viviparity was studied within an evolutionary context in the genus. Viviparity evolved within squamates (lizards and snakes) multiple times, especially in the lizard family Phrynosomatidae (Fitch, 1970; Shine, 1985; Blackburn, 2000). Twenty-nine out of sixty-nine Sceloporus species and six of

Figure 11: Geographic Ranges of Oviparous and Viviparous Phrynosoma.

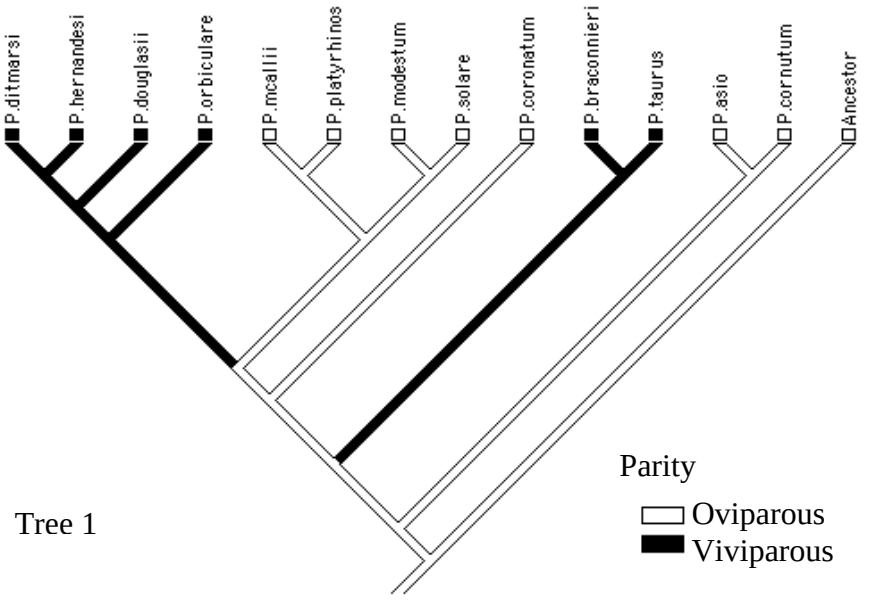
Maps showing geographic ranges of oviparous (top) and viviparous (bottom) species.

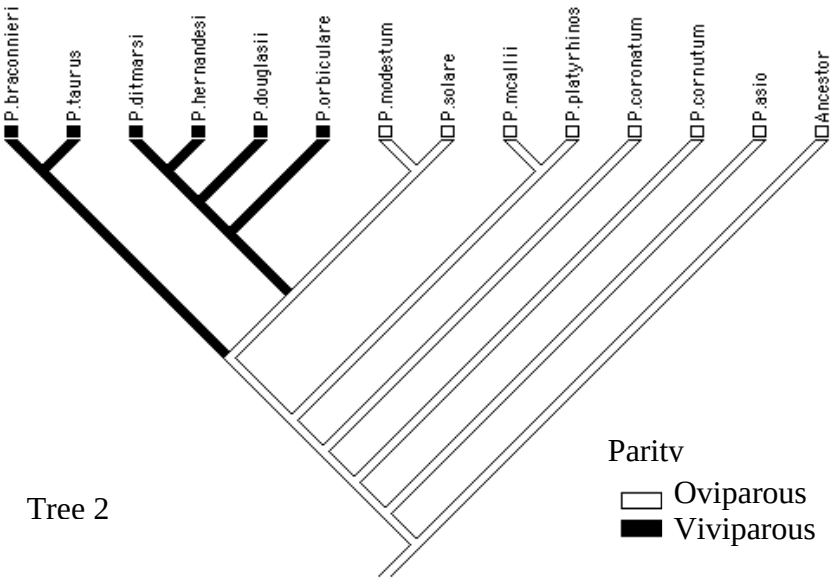


thirteen Phrynosoma species are viviparous (Montanucci, 1989; Sites et al., 1992; Mendez de la Cruz et al., 1998; Zamudio and Parra-Olea, 2000). Hodges (Chapter 1) investigated the monophyly of viviparous Phrynosoma species and determined that monophyly could not be supported by phylogenetic analyses. The best phylogenetic hypotheses chosen in these analyses suggest viviparity evolved twice in the genus (Figure 2). The cold-climate model has received broad support for explaining evolution of viviparity within squamates. Evidence supporting this model includes: 1. Viviparous species comprise a larger proportion of species at higher altitudes or latitudes; 2. Recent origins of viviparity are associated with recent invasions of higher latitudes and altitudes; 3. High latitudes and high altitudes are characterized by lower temperatures and distributions of viviparous species are highly correlated with low temperature; 4. Embryonic development slows or stops when embryos are exposed to cold temperatures favoring egg retention by females at higher latitudes and altitudes (Tinkle and Gibbons, 1977; Shine and Bull, 1979; Blackburn 1982; Shine, 1985; Andrews, 2000; Andrews and Mathies, 2000). Other hypotheses explaining evolution of viviparity in squamates invoke environmental unpredictability, extreme environments (e.g. flood-prone, dry, hot), or other specific characteristics such as an animal's general slow speed, single clutching or maternal brooding species (Shine, 1985). Two mechanisms have been suggested for evolution of parity: 1. Selection on embryonic development: embryos retained longer by females survive at a higher rate due to accelerated embryonic development; 2. Selection on embryonic mortality: increased egg retention equates to less time

Figure 12: Parity in Phrynosoma.

Mode of reproduction (parity) mapped onto two best phylogenies generated in a parsimony analysis of four mitochondrial genes and morphological characters (Chapter 1).





spent in a nest exposed to extreme environmental conditions or predators and mortality (Huey, 1977; Shine, 1985; Chalcraft and Andrews, 1999; Andrews, 2000;). I studied latitude, altitude and climate as correlates to viviparity in Phrynosoma using phylogenetic comparative methods to assess which variables, if any, might support the cold climate hypothesis for reproductive mode in Phrynosoma. Specifically, I tested whether viviparous Phrynosoma occur at higher altitudes or higher latitudes than oviparous species.

METHODS AND MATERIALS

A series of collecting trips was made throughout Mexico during the following time periods: 03 July - 28 November 1998, 8 April - 3 May 1999, 03 June - 15 July 1999, and 15 August - 01 September 1999. Additional specimens from Mexican museums, personal collections from Mexican biologists and data from the literature were also pooled (Appendix 2 for list of specimens). Data recorded from field specimens included live mass (to nearest 0.5 g using a Pesola scale) and size (snout-vent length and total length to nearest 1 mm), time/date; body (cloacal), air (at 1 m) and substrate (on ground at point of capture) temperatures (all using a cloacal thermometer to nearest 0.5 degree Celsius); location (geographic and microhabitat), any observed behaviors, and general climate information (cloudy, sunny, windy, etc). Lizard body temperatures were compared to air and substrate temperatures using Wilcoxon signed-rank tests.

Data collected after preservation and from museum specimens included snout to vent length (SVL), total length (TL), size of ovaries (length and width), number and size of follicles, number, length and width of eggs or embryos in oviducts, and size of testes (length and width). Reproductive measurements were taken to the nearest 0.05 mm using Vernier calipers under a microscope. Gonadal volumes were calculated using the equation for an ellipsoid ($4/3\pi a^2 b$, a = 0.5 x

gonad width and b = 0.5 x gonad length). The largest gonad was used to calculate volume in each specimen. Data from 351 specimens were included from both preserved and live specimens. Data from different sexes were compared using Mann-Whitney tests to determine if females were significantly different than males in SVL and weight. Reproductive data for each species were summarized monthly and plotted on graphs through time to show reproductive cycles and estimate the timing of major reproductive events. Climate data for locations near study sites or sites where specimens had been collected were obtained from the Servicio Meteorologico Nacional. Climate data included average monthly precipitation from 1941 to 1996, monthly average temperature, average maximum temperature and average minimum temperature from 1951 to 1980. Climate data were shown relative to reproductive events for each species.

Data from the literature were compiled on reproduction, altitude, and geographic ranges for all species. Reproductive data included information on mating, ovulation, oviposition or parturition, clutch or litter size, and testis size. Data recorded from specimens examined by Pianka and Parker (1975) were also used. Altitude records from specimens and published accounts were verified on 1:50,000 topographic maps produced by the Instituto Nacional de Estadística, Geografía e Informática (INEGI). On-site recordings of altitude and location were made with Global Positioning System (GPS) units and a Brunton altimeter; the recordings were later verified on topographic maps.

Midpoints of geographic ranges for all Phrynosoma species and five outgroup taxa were determined using data collected in this study and published locality data (Reeve, 1952; Stebbins, 1985; Conant and Collins, 1991; Baur and Montanucci, 1998). MapSource (2000) data and USGS and INEGI topographic maps were used to determine latitudinal and longitudinal coordinates for north, south, east and west limits for each species' range (using WGS 84 projections).

Range midpoints were calculated as simple distances halfway between range limits. Median altitudes were calculated from maximum and minimum altitudes throughout each species' range. Differences in maximum and median latitudes and altitudes of oviparous and viviparous species were analyzed using nonparametric, Mann-Whitney tests. Differences between oviparous and viviparous species also were analyzed by calculating independent contrasts (Felsenstein, 1985) using COMPARE (Martins, 2001) and performing Mann-Whitney tests on the contrasts. The two best phylogenies produced from a combined analysis of molecular and morphological data (Figure 12), were used in analyses (Chapter 1). Contrasts were assigned to an oviparous or viviparous group depending on the mode of reproduction present above the node where the contrast was calculated. If both reproductive modes were present, the contrast was not assigned to a group nor used in later comparative tests between oviparous and viviparous groups. Only contrasts that could be clearly assigned as oviparous or viviparous were used. Reconstructions of the latitudinal midpoint and maximum and median altitude of a hypothetical Phrynosoma ancestor's geographic range were performed using the generalized least squares (GLS) method of Martins and Hansen (1997) in COMPARE.

RESULTS

Species Accounts

Major reproductive events are summarized for all Mexican Phrynosoma species in Table 7. Species are treated separately in further detail in the following species accounts sections.

Table 7: Major Reproductive Events and Clutch or Brood Size Data

Summary of major reproductive events for Mexican Phrynosoma species. Details for each species are given in species accounts. All species except P. asio are viviparous. Clutch/litter size refers to total number of eggs or embryos counted in the oviducts of individuals for each species or number of offspring or eggs observed in accounts from the literature.

Species	Maximum Testis Size	Mating	Ovulation	Gestation or Oviposition
P. asio	May - June	April - July	April-June	June-Aug
P. braconnieri	Fall - Winter	Fall - Winter	June - Nov	June - Feb
P. ditmarsi	Sep - Nov	Sep - Nov	March - June	March - Aug
P. orbiculare	May-Aug	May-August	July - Oct	Aug - April
P. taurus	Nov - Feb	Nov - Feb	Jan - April	March - Aug

Species	Parturition or Hatchling Emergence	Clutch/Litter Size Mean	Range	SD	N
P. asio	Sep -Nov	18.1	10-28	5.6	9
P. braconnieri	Sep - Jan (April-May?)	8.9	6-13	1.6	14
P. ditmarsi	June - Aug	8.1	9-14	2	9
P. orbiculare	April - July	10.8	4-18	4	39
P. taurus	June - Oct	11.2	5-20	4.4	10

Phrynosoma asio

P. asio is an oviparous species found at four localities in Guerrero, Mexico, two north of the Rio Balsas and two south of the river. Elevation where lizards were collected ranged 540 – 1412 m; additional records show elevation range near sea level to 1500 m (Reeve, 1952; Davis and Dixon, 1961). Lizards were active during visits 20 April – 03 May, 02 - 03 July and 17-28 November. Museum collection records exist for specimens collected January – March, August - October and December, indicating the species may be active year round.

Baur (1979) reported the species exhibited extensive hibernation periods in captivity from September to April when exposed to cold climates. Lizards were found 0920 – 2039 hours. Lizards were surface active in spring and fall in the mornings (0920 – 1040 hrs) and evenings (1820 - 1840 hrs); juveniles were moving in midday, but adults were found in shaded locations by midday. Most lizards were found under various objects throughout the entire day in spring, summer, and fall including large boulders and rocks, Opuntia pads, and trash. Davis and Dixon (1961) found three P. asio on roads and nine "under bits of bark". Lizards were also found at the base of large plants or shrubs in the shade. One lizard was found 15 cm off the ground clinging to the stem of a shrub in the shadow of surrounding stems. At two locations near Zumpango del Río, P. asio was found in groups of two to four under the same boulders, sometimes with P. taurus. Lizards often were in the exact same location over several days.

This species had a strong proclivity for blood squirting: twelve percent of specimens caught by hand squirted blood, and many others exhibited ocular swelling and eye bulging indicative of onset of this behavior. Blood squirting was preempted in lizards displaying ocular swelling and bulging eyes by allowing lizards to rest quietly in the palm of the hand. Compared to other Phrynosoma species, the proportion of P. asio squirting blood in response to human capture is the highest within Phrynosoma (Hodges, in review).

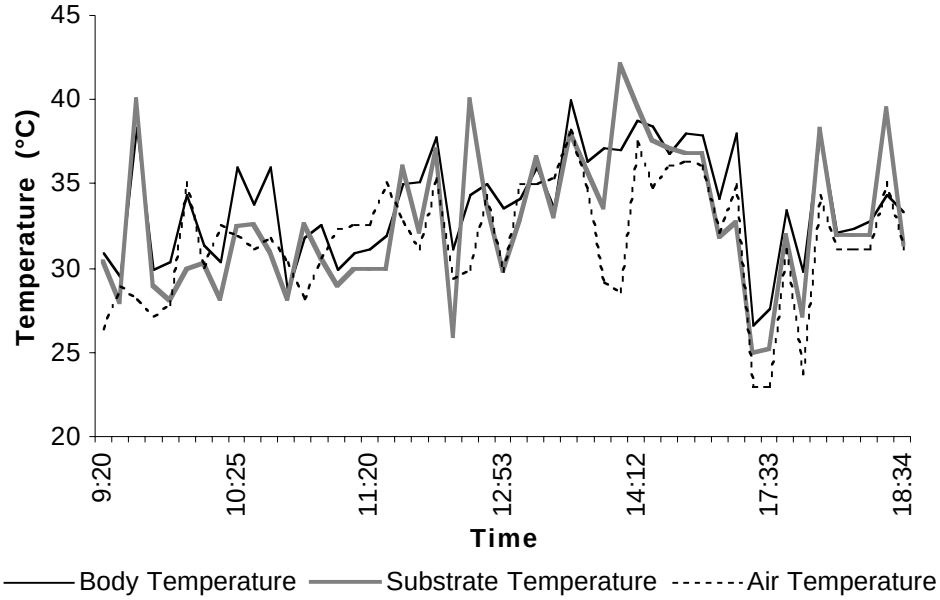
Body temperatures ranged from 26.6 °C in a juvenile female to 40 °C in an adult female. Body temperatures overall ($\bar{x}_{body}$ = 33.8 °C, SD = 3.24, N = 49) were significantly higher (Wilcoxon signed-rank, $p < 0.01$) than air temperatures

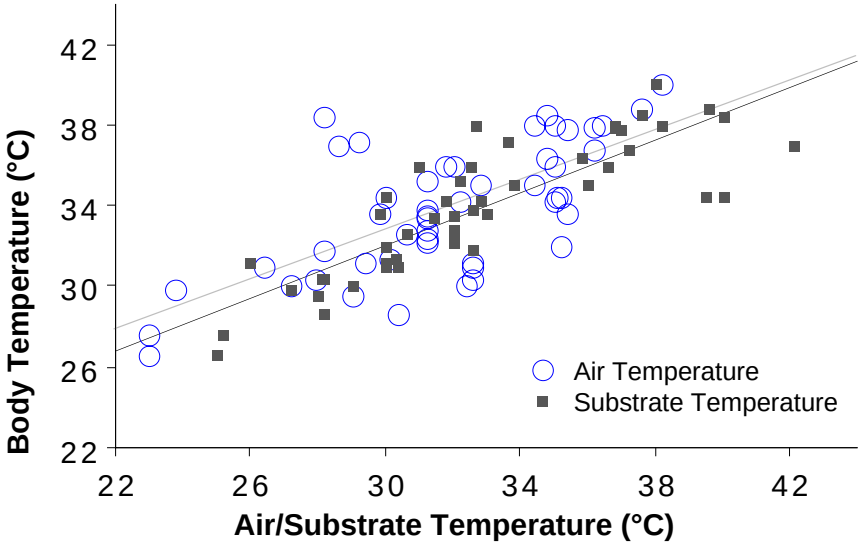
($\bar{x}_{air}$ = 31.8 °C, SD = 3.6, range = 23 – 38.2 °C, N = 49). Body and substrate temperatures were also significantly different (Wilcoxon signed-rank, $p < 0.01$, $\bar{x}_{substrate}$ = 32.7 °C, SD = 4.2, range = 25 – 42.1 °C, N = 49). Figure 13 shows regression slopes of body temperature with air (slope = 0.617) and substrate (slope = 0.653) temperatures suggesting P. asio actively thermoregulates and body temperatures more closely match substrate temperatures than air temperatures. Ballinger et al. (1998) reported 33 °C as the mean active body temperature from 31 specimens and the critical thermal maximum temperature for this species was 44.1 °C. No difference between adult female body temperatures ($\bar{x}_{body}$ = 34.03 °C, SD = 3.2, range = 27.6 - 40 °C, N = 26) and adult male body temperatures ($\bar{x}_{body}$ = 33.9 °C, SD = 2.8, range = 28.6 - 38 °C, N = 14) was detected. Captured adult females tended to be larger than males. Female SVL ($\bar{x}_{FSVL}$ = 101.8 mm, SD = 10.0, range 83 - 125 mm, N = 53) was slightly larger than males $\bar{x}_{MSVL}$ =101.2 mm, SD = 10.5, range 80 – 132 mm, N = 37), but the difference was not statistically significant ($p > 0.85$). Female mass ($\bar{x}$ = 53.8 g, SD = 13.0, range 32.0 – 78.5 g, N = 27) was significantly greater ($p < 0.01$) than male mass ($\bar{x}$ = 45.3 g, SD = 7.3, range 31.0 – 51.0, N = 25). Ballinger et al. (1998) reported body sizes 72 - 120 mm SVL ($\bar{x}$ = 90.1 mm) and masses 18 - 68 g ($\bar{x}$ = 39.0 g).

Male and female reproductive cycles appeared synchronized; maximum ovary and testis size occurred in late spring-early summer, May-June (Figure 14). Ovulation occurs over a longer period of time, April – July; one female collected 16 April contained 18 oviductal eggs. Davis and Dixon (1961) observed one

Figure 13: Phrynosoma asio Body, Air and Substrate Temperatures.

Top graph shows body temperature (solid line) with respect to air (dotted line) and substrate temperature (dashed line) of different lizards caught during the day. Bottom graph shows regression of body temperature on air and substrate temperatures (N = 49).





Top Line: Air Temperature Y = 14.234 + 0 .617 * X; R^2 = 0.466

Bottom Line: Substrate Temperature Y = 12.462 + 0.653 * X; R^2 = 0.714

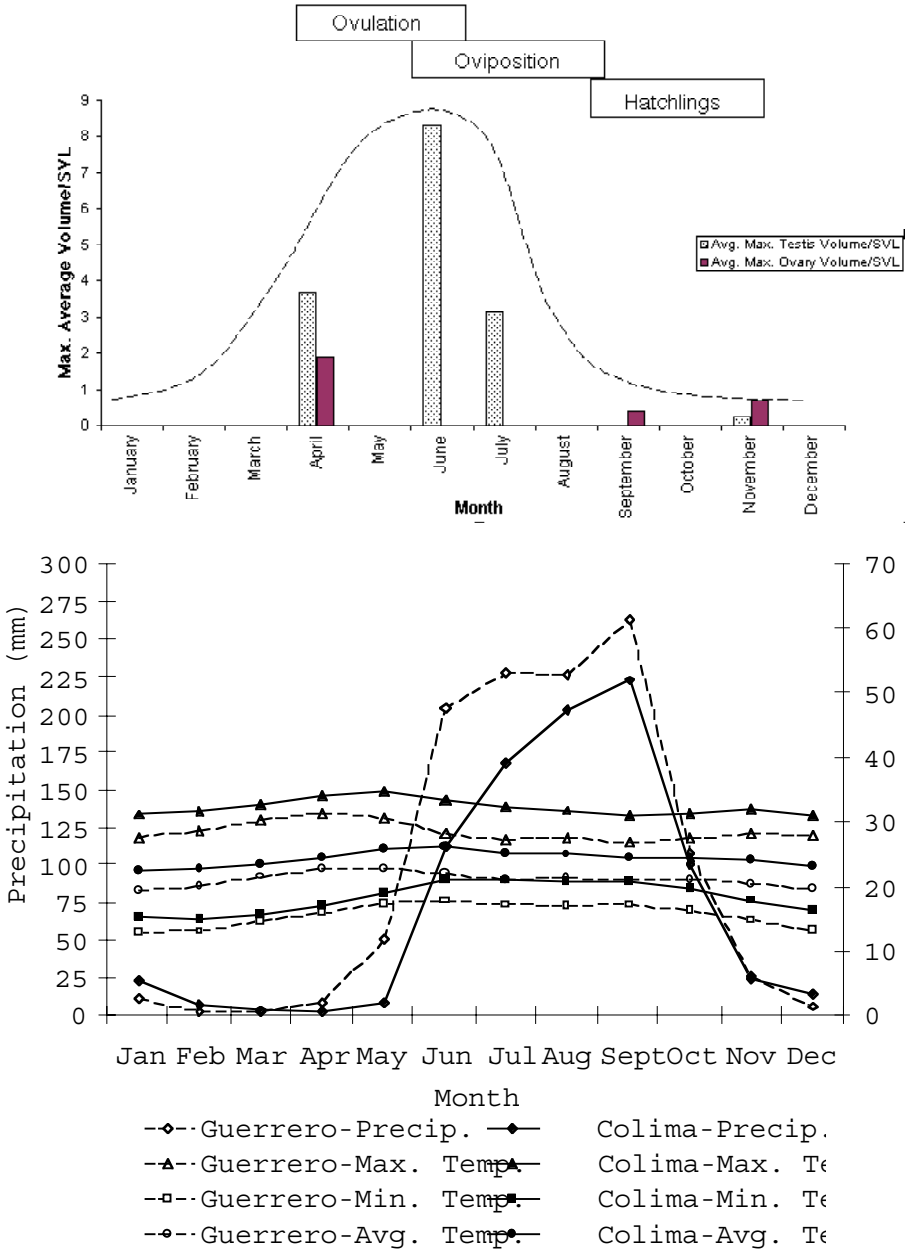
incidence of copulation on 26 June. The pair was kept in captivity until the female died 13 August while depositing a clutch of 21 eggs. Pianka and Parker (1975) also noted mid-August as the period of oviposition and mean clutch size of 16.9 (range 10-21). If females ovulate in mid-April, oviposition would start as early as June. Alvarez del Toro (1982) reported females lay 7-15 eggs, and hatchlings emerge after an 80-day incubation period when the rainy season subsides (September to October). Average clutch size from specimens in this study was 18.1 (SD = 5.6, range 10 - 28, N = 9), and the overall average from all reported studies was 17.2 (SD = 5.9, range 7 – 28, N = 12). The smallest reproducing female was 90 mm SVL. The smallest P. asio (SVL 31 mm) observed in this study on 17 November was presumed young of the year. Baur (1979) reported 26-day-old captive hatchlings with SVL of 32 and 34 mm. No juveniles observed earlier in April or July could be classified as hatchlings and were presumed hatchlings from the previous year. Hatchlings from females ovulating in April could appear by September as suggested by Alvarez del Toro (1982), and eggs oviposited mid-August may not hatch until November. No current evidence exists for females laying two clutches in a year despite the potentially long active season.

Phrynosoma braconnieri

Phrynosoma braconnieri was the most difficult species to locate. Lizards were found at two localities in Puebla: Cacaloapan and Chapulco (northwest and north of Tehuacan, respectively), on rocky hillsides at 1950-2100 m. The general habitat at Cacaloapan and Chapulco was dry, rocky, xeric short scrub under heavy

Figure 14: Major Reproductive Events: Phrynosoma asio and Climate Information

Top Graph: Male and female gonadal volume to size ratios plotted through time. Other major reproductive events are shown at the top of the graph. Bottom Graph: Rainfall and temperature patterns in Guerrero and Colima based on monthly averages.



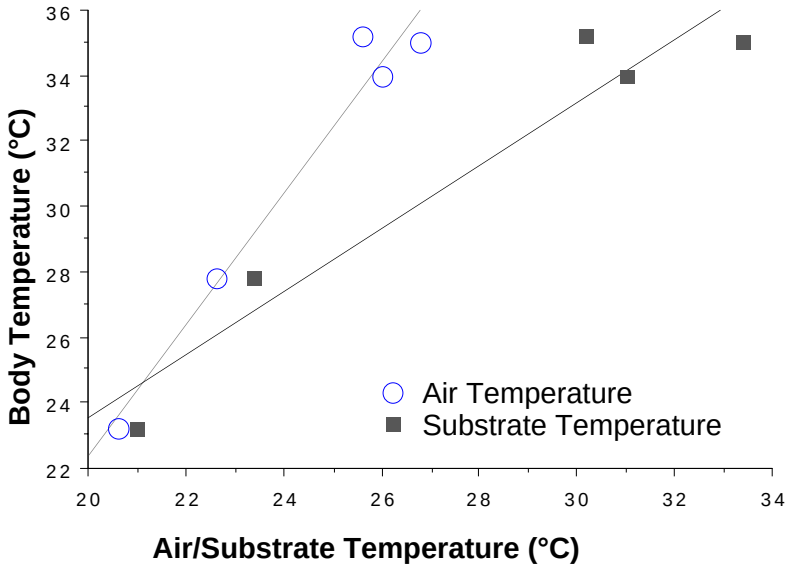
grazing by goats. Zamudio and Parra-Olea (2000) reported all known localities from museum specimens and the literature. Additional localities were obtained from Mexican specimens: Puebla: Amozoc, Valsequillo, San Lucas Teteletitlán; Oaxaca: Yosocuno. Several other locations are known by Mexican biologists, but were unconfirmed by specimens. Records from Oaxaca place this species at higher elevations than in Puebla, up to approximately 2500 m in pine-oak woodlands (Montanucci, 1979; Zamudio and Parra-Olea, 2000). The lowest recorded altitude is from the vicinity (5.6 km SSW) of Zapotitlán de las Salinas, Puebla, at 1494 m. At Cacaloapan, Puebla, P. taurus has also been recorded (Reeve, 1952); however, after four visits in different seasons, only P. braconnieri was found at this locality.

Specimens were found at Chapulco and Cacaloapan in June despite searching the same localities in April the same year. Activity appeared to be tied with summer rains; individuals were not active early in the year during hottest and driest periods, and they were often found when it was cool and cloudy during mid-day, 1030–1840 hrs. The majority of existing museum specimens were collected June – September. Temperature data for all lizards were pooled in the following Wilcoxon signed-rank tests because of low sample size for individual sexes (Figure 15). Active lizard body temperatures ($\bar{x}$ = 31.0 °C, SD = 5.3 °C; range 23.2 – 35.2 °C, N = 5) were significantly (p = 0.043) higher than air temperatures ($\bar{x}$ = 24.3 °C, SD = 2.6 °C; range 20.6 – 26.8 °C, N = 5), and significantly higher (p = 0.043) than substrate temperatures ($\bar{x}$ = 27.8 °C, SD = 5.3 °C; range 21 – 33.4 °C, N = 5). Mean adult female body temperature was

35.1 °C (N = 2) and male mean body temperature was 28.3 °C (N = 3). Adult female SVL was significantly larger than male SVL (p = 0.018). Adult females were 65.6 mm SVL on average (SD = 5.8, range 54 - 75 mm, N = 16) and weighed 18.8 g (SD = 0.35 g, range = 18.5 - 19.0, N = 2). Males were 58.3 mm SVL on average (SD = 3.9, range 52 – 63 mm, N = 7) and weighed 11.8 g (SD = 1.8, range 10.5 – 13g, N = 2).

Phrynosoma braconnieri is viviparous. Reproductive status of twenty-three females, including fourteen with oviductal embryos, was examined. Average litter size was 8.9 (SD = 1.9; range 6-13, N = 14). The smallest female with

Figure 15: Phrynosoma braconnieri Body Temperatures Regressed against Air and Substrate Temperatures.



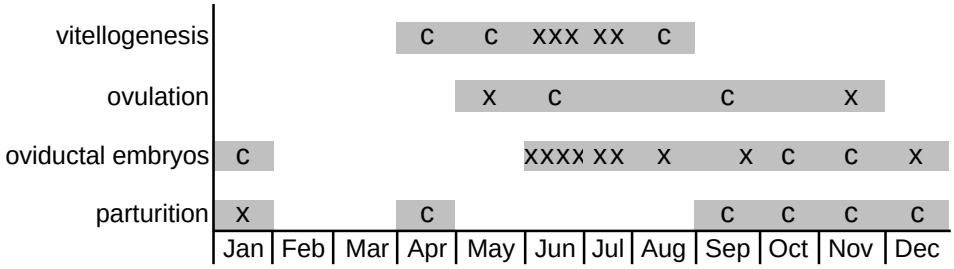
Top Line: Air Y = -17.635 + 2.001 X; R^2 = 0.966

Bottom Line: Substrate Y = 4.165 + 0.967 X; R^2 = 0.928

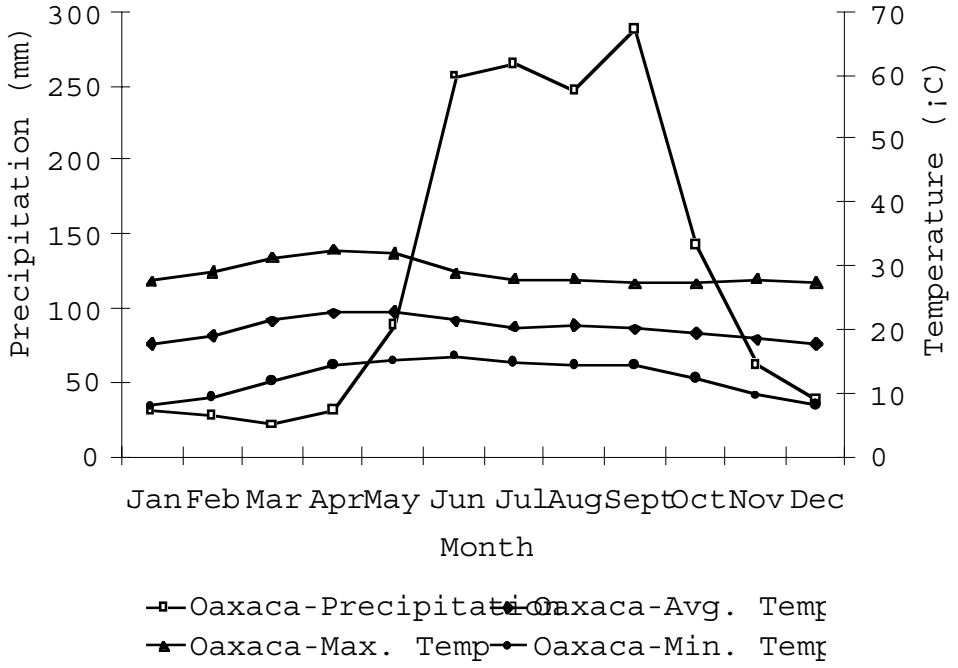
embryos was 54 mm. Females collected in June and July had developing embryos. Zamudio and Parra-Olea (2000) stated that they found females with developing embryos throughout the fall and winter. One specimen collected 15 September 1966 gave birth to young in captivity 24 January 1967, approximately 18 weeks later (Baur and Montanucci, 1998; Zamudio and Parra-Olea, 2000). If the female had mated just before capture, time from mating to parturition would match very closely the estimates for P. asio cycle from mating to hatchling emergence (6-7 weeks from mating to oviposition, 80 days incubation = 18 weeks). Alternatively, P. braconnieri may have a prolonged gestation of 18 weeks total or sperm storage for later fertilization post ovulation. One female collected 03 November 1970 had large (12 mm), yolky follicles but no embryos (Montanucci, 1979). Male sample sizes were too low to plot testicular size over time, but testes were enlarged in June, indicating they were sexually active. Gonadal sizes were much smaller in 2 specimens from July and August. These specimens were smaller in size (47 and 55 mm respectively) and were probably immature males. Montanucci (1979) and Zamudio and Parra-Olea (2000) suggested fall mating, implying fall may be the time for maximum testis size. The smallest individual collected was a female 38 mm SVL collected 23 June. This female could have been two months old, assuming she remained active postparturition and would indicate parturition in April, but could be earlier if it went dormant over the dry, hot period in spring. Data suggest potential for two separate parturition events, one in early spring with the onset of the rainy season, and one in late fall near the end of the rainy season (Figure 16).

Figure 16: Estimated P. braconnieri Reproductive Cycles and Weather Patterns in Oaxaca and Guerrero.

Top Image: Estimate of female reproductive events based on specimens and back calculations from known information. Vitellogenesis is the period of yolk formation, ovulation is the time ova move from ovary to oviduct, oviductal embryos refers to the period of time when embryos are developing in the oviduct, and parturition is the time lizards are born. Bottom Image: Rainfall and temperature patterns in Oaxaca based on monthly averages.



x=known data
c=back calculated



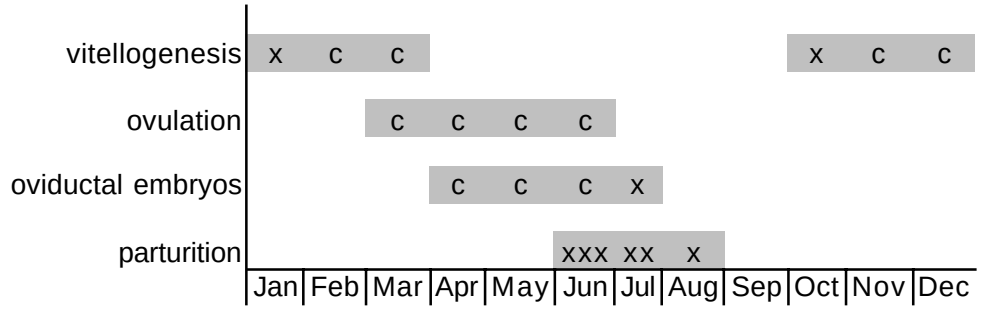
Phrynosoma ditmarsi

P. ditmarsi, a viviparous species, was found at two localities, Rancho La Palma and El Chorro, Sonora in July and August. Visits to both localities 7 - 12 June 1999 confirmed reports that the species is not active in the dry summer until rains begin (Montanucci, 1989b). Immediately before summer monsoon rains, regions were characterized by hot and dry climate (Figure 17) with little green vegetation except Opuntia dotting the hillsides. Perrill (1983) reported finding a 5 cm subadult P. ditmarsi in the Rio Yaquí drainage on 14 March 1983 at 1400 hrs.

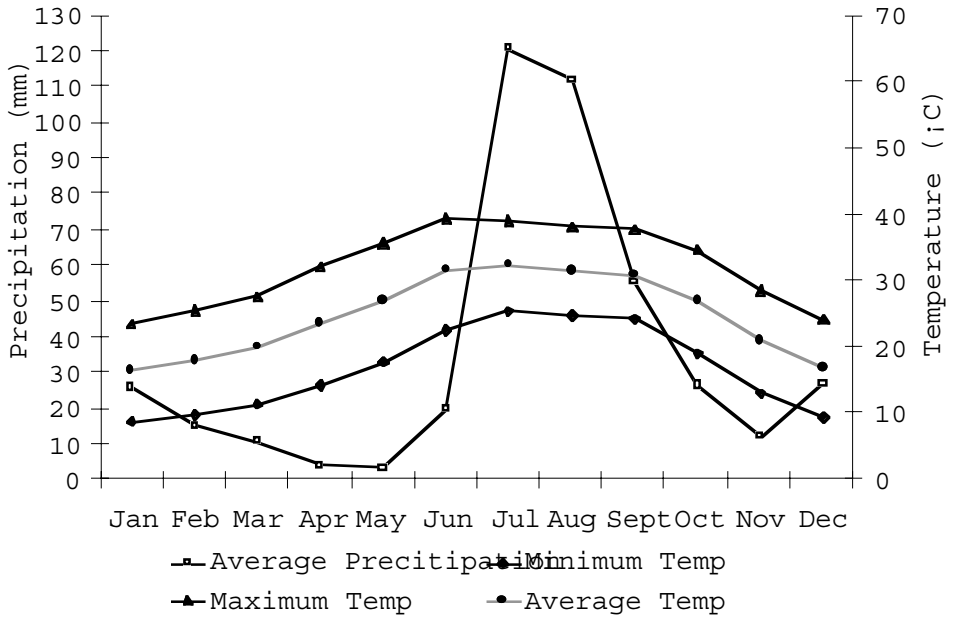
All lizards were found on high, rocky slopes 1040 – 1650 m elevation. Ground vegetation, particularly grasses, was important and lizards were often found in close proximity to vegetation clumps in open areas separated by stands of Quercus sp. Phrynosoma solare was also found below these hills in the surrounding lowlands and drainages at Rancho la Palma. Montanucci (1989b) noted captive P. ditmarsi often spent the night on or near grass clumps or rocks. For all age and size classes pooled, average body temperatures were above substrate and air temperatures. The highest body temperature was taken from a juvenile female at 39.2 °C, the lowest, 30.2 °C, was from another juvenile female (juvenile $\bar{x}$ = 33.9°C; SD = 3.0, N = 10). Adult female body temperature ($\bar{x}$ = 35.5 °C, SD = 1.4, N = 3) was slightly higher than male body temperature ($\bar{x}$ = 34.3 °C; SD = 0.71, N = 2). Three adults were tested for the ability to squirt blood at a potential canine predator; one did not squirt blood and two did (Hodges, in review). The largest female collected defecated considerable amounts of scat after being captured. The scat was comprised entirely of ants and weighed 3 g (8.2%

Figure 17: Phrynosoma ditmarsi: Major Reproductive Events with Associated Precipitation and Temperatures in Sonora.

Top figure shows estimated female reproductive cycle based on known data and back calculations from known events. Bottom graph shows rainfall pattern and temperature patterns in Sonora based on monthly averages.



x = known data
c = back calculated



of her total body weight post defecation). Adult P. ditmarsi were collected at Rancho la Palma from 0919 hrs to 1320 hrs in August.

Adult females were larger on average than males ($\bar{x}_{female}$ = 75.4 mm SVL, SD = 11.9, range 60 – 90 mm, N = 5: $\bar{x}_{male}$ = 60 mm SVL, SD = 4.6, range = 55-64 mm, N = 3). Lowe et al. (1971) reported an average 77 mm SVL for a pooled sample of three males and two females collected from the Sierra Manzanal, Sonora. Two neonates were observed at EL Chorro, Sonora, on 15 and 18 July 1998, 1000 - 1100 hrs ($\bar{x}$ = 24.5 mm SVL, <1 g) . I collected twelve other juveniles at Rancho la Palma on 21-29 August 1999, 0740 –1418 hrs. Juvenile males were distinguished from females by the presence of two enlarged postanal scales. No significant differences in SVL or mass were detected between juvenile males (N = 6, SVL: $\bar{x}$ = 29.3 mm, SD = 3.5, mass: $\bar{x}$ = 1.98 g, SD = 0.61) and females (N = 6, SVL: $\bar{x}$ = 29.7 mm, SD = 3.14, mass: $\bar{x}$ = 1.9 g, SD = 0.62). All Rancho la Palma juvenile lizards were larger than El Chorro neonates. They were probably born late July or August.

Male testis size and female ovary size in August were small. Lowe and Howard (1975) suggested male testicular cycle reaches maximum recrudescence in autumn and are at minimum size in mid-summer. Females in August contained an average of 31, very small ovarian follicles, 0.3 – 1.9 mm in diameter. The largest female was post-parturient, while other females' oviducts showed slight thickening, indicating they were becoming reproductive and may be reproducing their first year. Lowe and Howard (1975) reported 12.2 follicles, less than 4 mm in diameter from 4 females collected in October. Montanucci (1989) reported

breeding in late summer to fall for captive individuals. Lowe and Howard (1975) collected a gravid female on 13 July 1974 from Rancho la Palma, Sonora, and took it back to the lab. Parturition occurred on 23 July 1994; eight lizards and one stillborn were deposited. Live newborn lizards were on average 26.0 mm SVL and weighed 1.14 g. Montanucci collected both sexes of P. ditmarsi in August 1983 and kept them captive. A litter was produced on 06 July 1984. Montanucci (1989) reported litter size ranges of 1 - 13, and Zamudio (1998) reported a mean litter size of 8.4 from seven litters. Figure 17 represents current estimates of P. ditmarsi reproductive cycle. Parturition is timed with onset of summer monsoon rains in late June through October. Females were vitellogenic after October and probably would not ovulate until spring.

Phrynosoma orbiculare

Only three Phrynosoma orbiculare were collected at two localities. Efforts to collect this viviparous species were initially hampered by poor weather and thereafter not made because large numbers of specimens in Mexican museums were available. Two specimens were taken from pine-oak forest habitat approximately 15 km north of El Salto, Durango, on 29 August 1998, at 2300 m. One specimen was collected in thornscrub chaparral 1 km south of El Tablón (also called Vicente Guerrero Tablón), Hidalgo, on 23 September 1998, at 1850 m. All specimens were found late in the day (1625 – 1930 hrs) in cool weather (air temperature 19 - 26 °C). Reproductive and capture data from 151 museum specimens and additional literature sources are summarized. Specimens have been collected from all months of the year, except December, from altitudes of 1100 –

3350 m in a variety of habitats ranging from grasslands to thornscrub chaparral to oak and pine forests throughout the central Mexican plateau. Two reports of specimens taken at lower elevations appear to be errors. Smith and Laufe (1945) dismissed a record from 185 m in Guerrero. The collection information from a specimen reportedly found at 246 m at Chalchihuites, Zacatecas, is in error because this site is at approximately 2200 m. (The original collection locality says “800 ft”, and perhaps should have been “8000 ft”.) Seasonal inactivity may coincide either with cooler temperatures or dry conditions. Several collection notes mentioned that animals were found following rain after prolonged periods of dry weather.

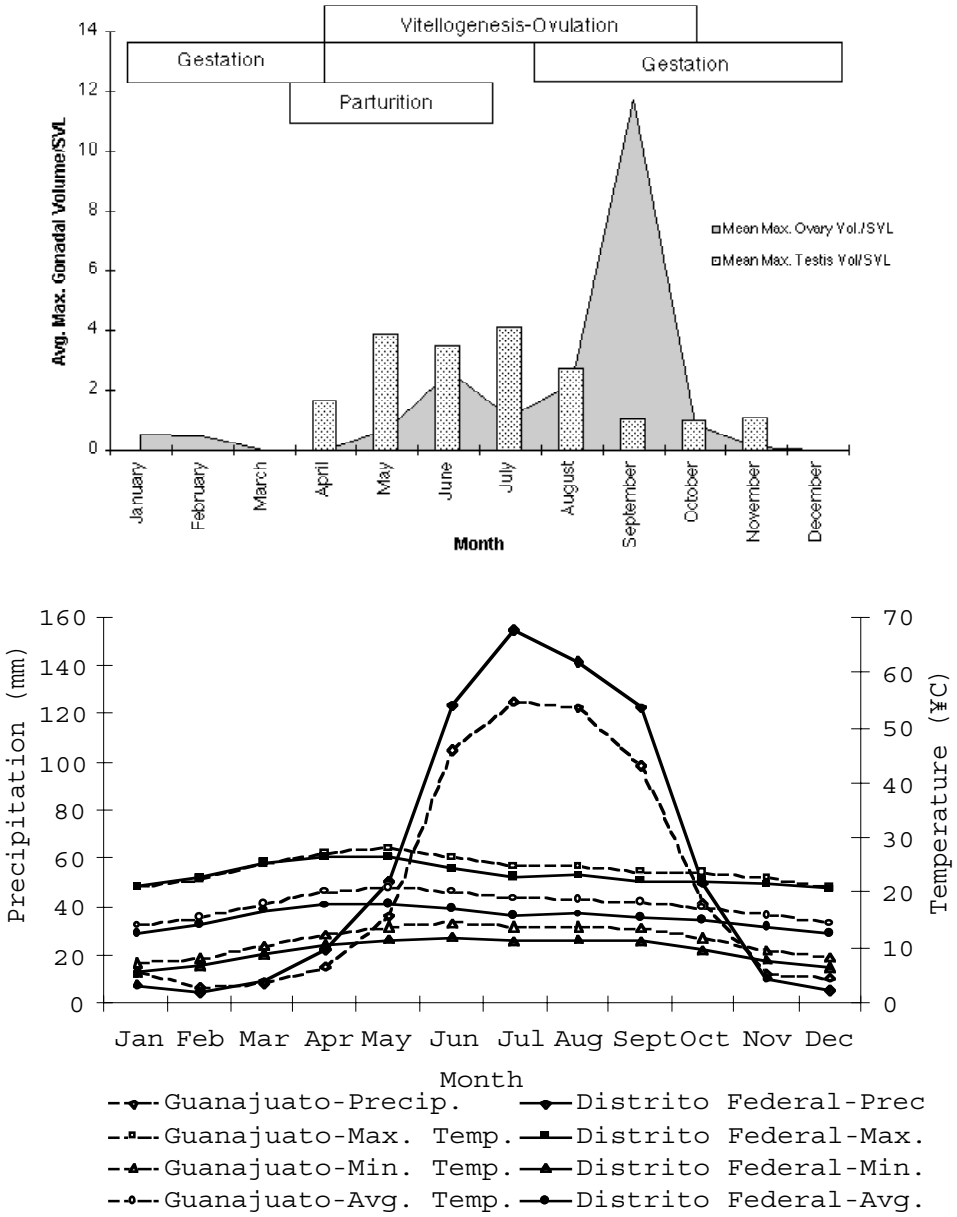
Females were separated into two classes, juveniles and adults, based on reproductive status. Adult female size was on average 76.2 mm SVL (SD = 11.3, range 54 – 110.5 mm, N = 91). Juvenile female size was 44.9 mm SVL (SD = 6.3; range 31 - 51.5 mm, N = 9). This class designation is somewhat arbitrary because females in the size range 50-70 mm SVL showed considerable variation in reproductive condition. Further studies should clarify age and size of first reproduction for different populations of this widespread species. Specimens indicated females may take one full year to become reproductive and would not reproduce until the second summer after parturition. However, Baur (1987) noted a single captive female produced four young eleven months postparturition. Males were divided into two size classes using gonadal condition as a marker for maturation. Adult male size was 74.3 mm SVL on average (SD = 9.4, range 55 -

101 mm, N = 42); juvenile males were 42.9 mm SVL on average (SD = 8.2, range 31 – 52 mm, N = 9).

Phrynosoma orbiculare is viviparous and gestation occurs through the winter with parturition in early spring to (Figure 18). Mating was observed in May when male testis size was near maximum. Montanucci (1989) reported mating activity in captive individuals from August to November. Male gonad size peaked earlier than female gonad size. Adult postparturient females contained yolked follicles that gradually enlarged from late May through September. Gestation occurs from July or August to the following spring. Females with early stage embryos were collected in late July to late October, and females with well-developed embryos were collected from late January to mid-March (Hernandez-Ibarra et al., 2000). Parturition appeared timed with the beginning of summer rains after gestation over winter (Figure 18) . The smallest female with embryos was 67 mm SVL, though one female 54 mm SVL collected in October showed evidence of vitellogenesis. Average litter size was 10.8 (SD = 4.0, range 4 – 18, N = 39) with an average embryo length of 9.7 mm (SD = 1.7, range 7.2 - 14.7 mm, N = 24). Hernandez-Ibarra et al. (2000), reported an average length of 11.3 mm from 17 well-developed embryos. Females with embryos did not show any sign of vitellogenesis. Only postparturient females showed signs indicating this species has only a single brood each year. The smallest specimen, 31 mm SVL, was collected on 15 July and was probably born in June. Castillo-Olivares (1993) reported seeing the first young of the year on a study site in Veracruz in May, while other age classes were active from February through September.

Figure 18: Phrynosoma orbiculare Reproductive Cycles and Climate Patterns.

Top graph shows ovarian and testicular sizes through time with corresponding data on gestation and parturition of embryos. Bottom graph shows precipitation and temperature data for two localities where P. orbiculare have been collected.



Phrynosoma taurus

A total of 42 specimens was examined from field and museum specimens. Phrynosoma taurus is a viviparous species and was found in the field at two locations: west of Zapotitlán de las Salinas, Puebla, at 1600 m, and several sites around Zumpango del Río, Guerrero, at 1100 - 1400m. Both locations were arid, rocky thornscrub habitats. Phrynosoma taurus was found in syntopy with P. asio at two sites near Zumpango del Rio, under the same rocks and boulders at times. Activity was different between Puebla and Guerrero. In Puebla, lizards were only found after summer rains began; earlier visits (in April) before rains began produced no specimens. Notes from other specimens in June stated they were collected on cloudy, cool days. However, in Guerrero, it was wetter and cooler in April and June, and lizards were active; a visit in November produced no specimens. Zamudio and Parra-Olea (2000) listed previously known localities from museum specimens. Additional localities from specimens in Mexican collections include: Puebla: San José Axuxco (ca. 1000 m), Cantera de Tlayua in Municipio Tepexi de Rodriquez, Santo Domingo Huehuetlan, 3 km NE Piaxtla (1260 m), and between Tlalcualpican and Limones (1050 m) in Municipio Tlalcualpican; Guerrero: Cerro Tepetlayo, Cerro Tepehuixtle (both near Zumpango del Río). Elevation range for all specimens was 1000 – 1900 m.

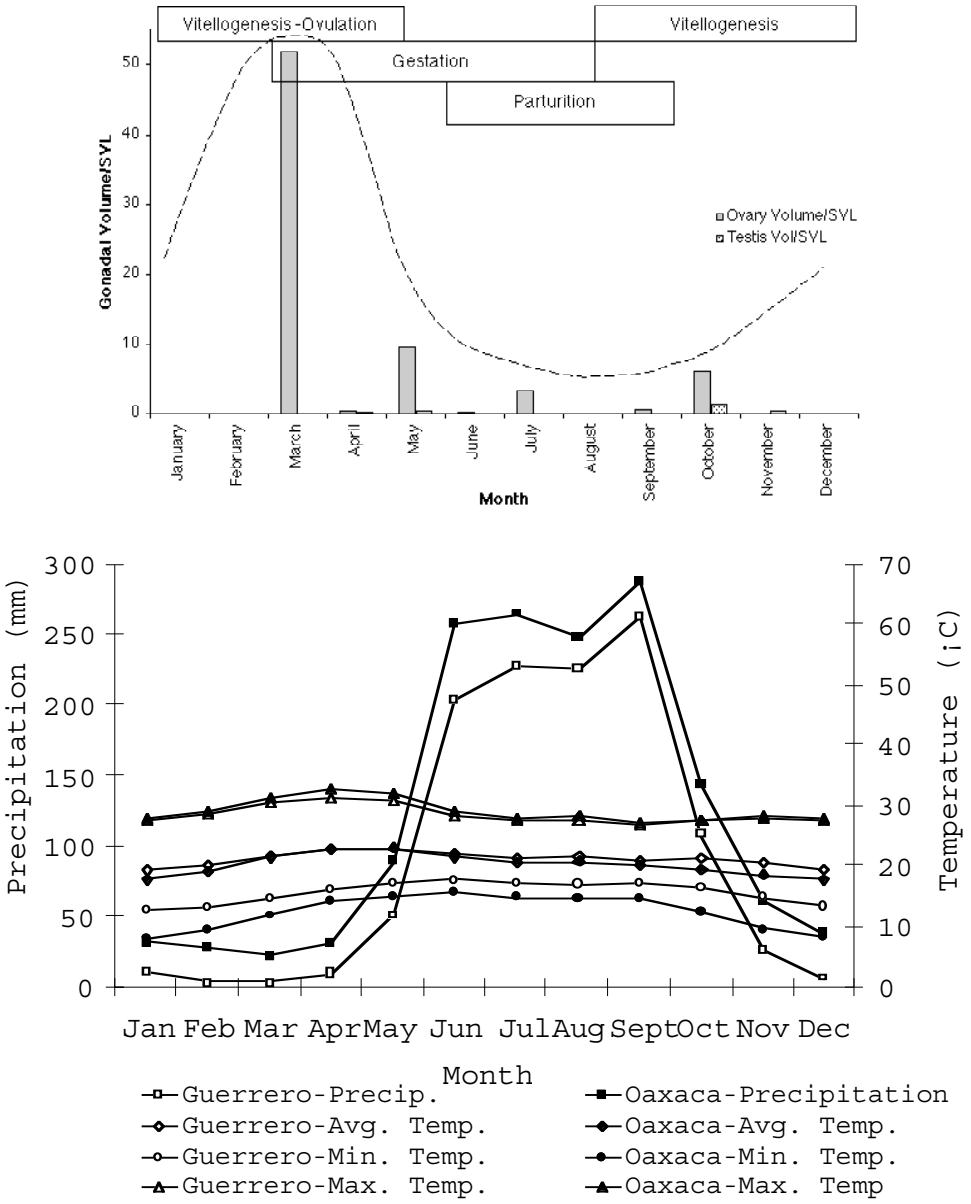
Individuals were collected throughout the day between 0925 to 1830 hrs, some basking until 1100 hrs and after 1730 hrs. Other specimens were found under rocks or in the shade of bushes or boulders for the rest of the day, while some individuals could be found under rocks any time of day. As noted earlier,

Phrynosoma taurus occurred under the same rocks and boulders as P. asio at sites near Zumpango del Río. Females were found throughout the day when air temperatures were between 25.2 °C and 37.2 °C, but males were only found at mid-day (1244 - 1610 hrs) at temperatures between 32.4 °C and 38.4 °C. Mean body temperature of all active lizards was 35.8 °C (SD = 2.4, range 32.4 - 40 °C, N = 20). Males ($\bar{x}$ = 38.5 °C, SD = 2.2, range 36 – 40, N = 3) were hotter than females ($\bar{x}$ = 35.3 °C, SD = 2.0, range 32 – 38, N = 13), but the difference in body temperatures was not significant ($p > 0.17$). Male SVL ($\bar{x}$ = 70.8 mm, SD = 9.4, range 60-88 mm, N = 6) was smaller than adult female SVL ($\bar{x}$ = 74.8 mm, SD = 7.4, range 60-88), but the difference was not significant ($p >0.2$). Female body mass ($\bar{x}$ = 32.6 g, SD = 10.3, range 14-54, N = 12) was greater than male body mass ($\bar{x}$ = 13.3 g, SD = 4.0, range 11-18, N = 3) due to the large proportion of females carrying embryos, but the difference was not significant ($p > 0.10$) due to low sample size. The largest lizards collected were a male and female, SVL = 88 mm. The smallest juvenile collected was a female, 27 mm SVL, on 02 July.

One female collected on 01 February was kept captive until it died 30 March and had large ovarian follicles (6.7 – 8.4 mm diameter). One female collected 01 May had large follicles (5.5 – 7.0 mm diameter) indicating that ovulation occurs from March through May (Figure 19). Females were gestating in April and carried an average 11.2 embryos (SD = 4.4, range 5-20). Embryos from females collected in April and May varied considerably in stage of development from primarily yolk to well developed embryos with little remaining

Figure 19: Phrynosoma taurus Reproductive Cycles and Climate Information

Top Graph: Testis and ovarian cycles for P. taurus are shown through time with major female reproductive event shown at the top. Bottom Graph shows climate data for two localities where the species is found.



yolk. The smallest female with embryos was 60 mm SVL. Davis and Dixon (1961) found one sexually inactive female on 17 June that measured 52 mm SVL. During gestation, no vitellogenesis or other follicular development was apparent in any specimen. The smallest juvenile collected on 02 July was probably born early in June. Postparturient females were collected July to October. Zamudio and Parra-Olea (2000) found postparturient females from mid-June to mid-August.

Parturition probably occurs from the end of May or June through August or September, coinciding with the rainy season in areas the species is found. Once parturition occurred, vitellogenesis began. Information on male testis cycle was minimal, but the male with the largest testis size was collected in late October. With ovulation occurring in late winter to early spring, male testis cycle may peak near the same time or earlier in late fall, with mating sometime between November and February. Although temperatures in these months may be colder, average maximum monthly temperatures are well within the active temperature range observed in the lizards (Figure 19).

Range and Elevation of Oviparous and Viviparous Species

Maximum elevation and latitude were not significantly different between oviparous and viviparous species. Elevation data for all species are shown in Table 8 along with data on estimated geographic coordinates for the maximum latitude and midpoint of each species' range. Viviparous species occur at an average median elevation of 1820 m and at 33.211° north latitude; oviparous species occur at an average median elevation of 826 m at 35.596° north latitude.

Comparing minimum, maximum, and median altitude in each group using nonparametric Mann-Whitney tests showed viviparous species occur at significantly higher minimum and median altitudes than oviparous species (p = 0.025); but not maximum altitude (p = 0.1061). Comparing geographic ranges of oviparous and viviparous species showed these groups did not significantly differ

Table 8: Elevation and Range Midpoints Estimated for each Phrynosoma Species

Estimates calculated for each Phrynosoma species elevation and geographic range are grouped by mode of reproduction. Results of Mann-Whitney test are shown at the bottom and compare raw data for oviparous and viviparous species.

Oviparous Species

	Altitude Range (m)		Altitude	Midpoint (Lat/Long)		Maximum
	Minimum	Maximum	Median	North	West	Latitude
P. asio	0	1500	750	16.94693	98.16383	19.31834
P. cornutum	0	1800	900	31.66733	101.599	39.98611
P. coronatum	0	2000	1000	31.06013	115.9727	40.5251
P. mcallii	0	520	260	32.61809	115.7591	33.91988
P. modestum	200	2200	1200	28.05308	104.7382	37.42121
P. platyrhinos	-100	1980	940	37.39229	114.8604	44.28814
P. solare	0	1460	730	28.66027	111.0507	34.26958
Average	14	1637	826	29.48545	108.87768	35.67548
StDev	90	561	296	6.31800	7.33800	8.08200
StError	34	213	112	2.38800	2.77400	3.05500

Viviparous Species

P. braconnieri	1500	2500	2000	18.15401	97.08372	19.28614
P. ditmarsi	1000	1650	1325	30.51077	110.3686	30.77452
P. douglasii	700	3500	2100	41.49808	115.4711	49.31574
P. hernandesi	*	*	*	37.47903	106.7634	50.12423
P. orbiculare	1100	3350	2225	22.67578	101.5247	30.48194
P. taurus	1000	1900	1450	18.37331	98.50814	19.28614
Average	1060	2580	1820	28.11516	104.95327	33.21145
StDev	288	833	405	9.96200	7.19000	13.75900
StError	129	372	181	4.06700	2.93500	5.61700

Mann-Whitney test of significance between oviparous and viviparous

0.0025	0.1061	0.0025	0.9452	0.2949	0.5338

* P. hernandesi altitude is contained within P. douglasii

in geographic latitude either at the midpoint or at the maximum latitude of species' ranges. Coordinates placed the average midpoints of the geographic ranges of oviparous and viviparous at nearly the same latitude (Table 8). The midpoint of oviparous species was placed in the state of Sonora, Mexico, near the border of Chihuahua, 215 km south-southeast of Douglas, Arizona. The midpoint range of viviparous species was located in Chihuahua near the Coahuila border, 169 km south-southwest of Presidio, Texas. Reconstructed values for a hypothetical Phrynosoma ancestor (Table 9) placed it either northeast of Durango, Mexico, on the border of Durango and Coahuila or further south in Zacatecas, Mexico, at a median altitude between 1653 m and 1747 m.

Calculated independent contrasts are shown in Table 10 for each phylogeny used from Figure 1. The contrasts were analyzed with nonparametric, Mann-Whitney tests and showed all comparisons of altitude and latitude were not significantly different between the two modes of reproduction.

Table 9. Estimate of Ancestral Phrynosoma Altitude and Range

Using phylogenies from Figure 1, values for the altitude and latitude of the Phrynosoma ancestor were calculated.

	Altitude			Range	Midpoint	Maximum
Tree1	Minimum	Maximum	Median	North	West	Latitude
Estimate	366	1940	1747	25.0144	103.2632	30.22121
Tree2						
Estimate	54	1689	1653	23.6526	102.4448	28.57865

Table 10: Independent Contrasts of Altitude and Geographic Range

Independent contrasts for maximum, minimum and median altitudes and geographic range midpoint and maximum latitude for oviparous and viviparous species in Figure 1.

Tree 1 Contrasts

Oviparous Contrasts

	Altitude Range		Altitude	Geographic Midpoint		Maximum
	Minimum	Maximum	Median	North	West	Latitude
	0	-16.104844	-16.104844	-0.79023	-0.184408	-1.109504
	7.624929	-111.32396	-111.32396	-0.36403	0.068523	-0.790572
	11.45197	42.372277	42.372277	-0.03477	-0.361452	0.180462
	-10.68052	-25.067326	-25.067326	0.479075	0.477772	0.306532
	-29.26585	-21.87139	11.667347	-0.00931	0.437332	0.153807
	-44.50174	-35.050912	-11.517175	-0.07309	-0.408126	-0.051071
Average	-10.895	-27.841	-18.329	-0.1321	0.00494	-0.218391
StDev	22.036	49.259	51.632	0.4215	0.3887	0.587
StError	8.996	20.11	21.079	0.1721	0.1587	0.2396

Viviparous Contrasts

	34.50328	41.403934	37.953606	-0.01513	-0.098294	0
	22.36068	-57.392411	-20.49729	-0.51938	0.268717	-1.442242
	8.080705	-102.55762	-45.285618	-0.49216	-0.562554	-0.459949
	-23.03833	-31.513532	-26.488088	1.038933	0.689959	1.052686
Average	10.658	-37.515	-13.579	0.003063	0.07446	-0.212376
StDev	24.795	60.249	35.942	0.7284	0.533	1.036
StError	12.398	30.125	17.971	0.3642	0.2665	0.518

Mann-Whitney Tests

	0.1714	0.6095	0.6095	0.9999	0.9143	0.9143

Tree 2 Contrasts

Oviparous Contrasts

	Altitude Range		Altitude	Geographic Midpoint		Maximum
	Minimum	Maximum	Median	North	West	Latitude
	11.45197	42.372277	42.372277	-0.03477	-0.361452	0.180462
	7.624929	-111.32396	-111.32396	-0.36403	0.068523	-0.790572
	60.425	58.948639	24.487473	-0.7292	-0.790276	-0.742797
	28.91067	-2.11258	-21.087826	-0.0899	-0.488515	-0.395298
	13.22835	11.233549	2.551243	-0.07818	0.653283	-0.147993
	-6.642856	-23.408341	-19.048364	-0.83254	-0.531501	-1.149711
Average	19.181	-4.048	-13.675	-0.3548	-0.2645	-0.50765
StDev	23.195	60.405	53.815	0.3515	0.5076	0.4823
StError	9.469	24.66	21.97	0.1435	0.2072	0.1969

Viviparous Contrasts

	34.50328	41.403934	37.953606	-0.01513	-0.098294	0
	22.36068	-57.392411	-20.49729	-0.51938	0.268717	-1.442242
	8.080705	-102.55762	-45.285618	-0.49216	-0.562554	-0.459949
	-23.03833	-31.513532	-26.488088	1.038933	0.689959	1.052686
Average	10.477	-37.515	-13.579	0.003063	0.3557	-0.21238
StDev	24.816	60.249	35.942	0.7284	0.3503	1.036
StError	12.408	30.125	17.971	0.3642	0.1752	0.518

Mann-Whitney tests

	0.9143	0.3524	0.6095	0.4762	0.1143	0.7619

DISCUSSION

Mexican Phrynosoma exhibit a wide diversity of reproductive patterns ranging from spring mating with summer/fall hatchling emergence to fall/winter mating with spring/summer parturition. Viviparous species apparently time parturition with the beginning to middle of the rainy season. The exception may be P. braconnieri, which showed parturition in the winter (at least in captivity) after the rainy season had ended. Phrynosoma asio, an oviparous species, deposits its eggs at the beginning of the rainy season, but hatchling emergence does not occur until the end of the rainy season. Reproductive cycles of P. asio were nearly identical to P. solare; both are oviparous species living in areas that experience similar summer rainfall patterns (Blount, 1929). Oviposition at the beginning of the rainy season would guarantee warm, moist soil for lizards to lay their eggs in required for proper embryonic development. Parturition for viviparous species at the beginning or during the rainy season would reduce physiological impacts of water loss during parturition of embryos. All Phrynosoma species live in arid to semi-arid environments and water could influence the timing of reproductive events for these species. Phrynosoma obtain daily water from licking dew off of rocks or vegetation and from their food. However, water loss in viviparous species is great during parturition. Viviparous species may require an external water source rather than relying on metabolic water to compensate for water loss during parturition. Embryos developing in eggs laid in a nest also require external water during development provided by surrounding soil in the nest. Parturition and oviposition at the onset of or during

rainy periods would guarantee external water sources that are not available during other times of the year.

Viviparous species exhibit more variation in reproductive cycles than oviparous species. Phrynosoma ditmarsi and P. orbiculare show asynchronous annual parturition with only minor overlap in mid-summer. Phrynosoma ditmarsi annual reproductive pattern more closely matches the "typical short-horned viviparous pattern" reported in Zamudio and Parra-Olea (2000) which includes P. douglasii and P. hernandesi. Phrynosoma ditmarsi is more closely related to these species; P. orbiculare is the sister taxon to all three species. Though the P. orbiculare annual cycle is unlike other members of its clade, it is similar to other viviparous species in its geographic range (Ballinger, 1973; Mendez de la Cruz et al., 1988; Guillette and Mendez de la Cruz, 1993; Mendez de la Cruz et al., 1995). A consistent pattern between P. ditmarsi and P. orbiculare is the timing of parturition near the beginning of the rainy season. The onset of rains varies annually depending on geographic location and may explain asynchrony observed in species with the same mode of reproduction.

Parturition in Phrynosoma taurus occurs near the onset of summer rains also, but P. braconnieri parturition may occur both during and at the end of the rainy period. Annual reproductive pattern in P. braconnieri is more similar to the pattern found in the short-horned lizards, P. ditmarsi, P. douglasii and P. hernandesi. If parturition in this species occurs in the early spring, it would include the very early onset of summer rains, but would not overlap with parturition in P. taurus. Mid-summer parturition for P. braconnieri does not occur

in Pueblan populations since all females collected from these localities in June and July contained very early stage embryos. Phrynosoma taurus did contain fully developed embryos in June in Guerrero and were postparturient in both Puebla and Guerrero in September and October. These species are sister taxa and found in near sympatry and experience the same climate patterns. Shifts in parturition in these closely related taxa would allow both species to undergo parturition during different parts of a rainy season and concomittantly provide temporal separation between closely related neonates.

Two other sympatric Mexican species exhibit temporal separation of young. Phrynosoma taurus occurs in syntopy with P. asio in Guerrero. Emergence of P. asio hatchlings occurs later than appearance of P. taurus neonates. However, P. asio must first lay its eggs in warm, moist soil, which would not be available until rains begin. Oviposition and parturition occurs at the same time in the two species, but the time required for development of P. asio eggs temporally separates the presence of both species' young and would effectively reduce interspecific competition for food resources. Timing of reproductive events for different modes of reproduction is tied to rain events, which also reduces competition between related taxa.

Comparisons between oviparous and viviparous species and their geographic ranges and altitudes suggest altitude is more important than latitude with respect to reproductive mode. Maximum latitudes and midpoint latitudes are not significantly different between oviparous and viviparous species. Within the altitude gradient of current species' distributions, minimum and median altitudes

were significantly different between oviparous and viviparous species, but not maximum altitude. These results conflict in part with predictions from the cold-climate hypothesis, which states viviparous species occur at higher latitudes and altitudes than oviparous species because of colder temperatures found in these localities (Shine, 1985). Viviparous species did occur at higher minimum and median altitudes, but differences in maximum altitudes were not statistically significant. Although no difference in maximum altitude was evident, differences in minimum and median altitudes suggests oviparous species can occur at both high and low elevations, and that viviparous species are limited in their geographic range to high elevations. The cold-climate hypothesis states oviparous species should not occur at high altitudes or latitudes because of constraints acting on embryonic development or nest mortality. However, oviparous Phrynosoma are not limited geographically in latitude or altitude compared to viviparous Phrynosoma, which are restricted to higher altitudes. The elevation limitation imposed on viviparous Phrynosoma may be an artifact of the phylogenetic history of the genus.

Species may share characteristics because they are related, not because of causal ecological circumstances. Independent contrasts consider the role phylogenetic relationships may play in species traits. When oviparous and viviparous contrasts were compared, no differences at all were seen in altitude or latitude between contrasts grouped by reproductive mode. When phylogenetic relationships of species are incorporated into the analysis, differences attributed to reproductive mode disappears suggesting historical events may be responsible for

restricting viviparous species to higher altitudes rather than evolutionary forces. An historical artifact may explain why viviparous clades arose at the same time species invaded high altitudes. However, since viviparity has evolved at most twice in the genus, no amount of difference between the groups may be detectable. A better test for the cold-climate hypothesis and its predictions about viviparity evolving with high altitude or latitude would consider numerous closely related taxa that exhibit different reproductive modes.

Phrynosoma is a relatively old genus that diverged from other phrynosomatid genera by the late Oligocene or early Miocene (Montanucci, 1987; Etheridge and de Queiroz, 1988). A midpoint for the geographic range and altitude of the ancestor to Phrynosoma places it in north central Mexico at a median altitude between 1653 –1747 m. Although independent contrasts have been recommended and used in comparative studies of species ranges and habitat characteristics (Garland et al., 1992; Lovegrove, 2000; Canterbury, 2002), estimating ancestral range and altitude with other methods (Ronquist, 1997) may give different estimates.

In the late Oligocene, western North American mountain ranges were uplifted (King, 1958; Barnovsky and Labar, 1989). This uplift could have been a vicariant event quickly separating two lineages differing in reproductive mode. Benefits of viviparity during this time period of unstable climatic conditions were probably important in early divergence of oviparous from viviparous Phrynosoma clades rather than modern climatic conditions. Fossil material for the short-horned, viviparous clade (P. douglasii, P. hernandesi, P. ditmarsi, and P.

orbiculare) dates these species to mid-Miocene (Robinson and Van Devender, 1973), which places the origin of this clade to the early to mid-Miocene or before. Evolution of this clade was probably associated with mountain building and associated habitats that began in the Oligocene (Zamudio et al., 1997).

Viviparity evolved early in the history of the genus, but has been maintained in Phrynosoma in recent times when viviparity may not have the same advantages as it previously did. Once viviparity has evolved, it is difficult to reverse to oviparity (Lee and Shine, 1998). Currently, viviparity appears to be advantageous at two levels. Viviparity may be more flexible than oviparity: closely related viviparous species in sympatry coexist by shifting reproductive patterns so that neonates are born temporally separated, which may reduce competition. Viviparous and oviparous species of Phrynosoma occur in syntopy and time reproductive events that maximize benefits of the rainy season. Oviparous species may have invaded habitats with viviparous species because they have temporally separated emergence of neonates. Oviparous species are restricted in reproductive timing by the need for warm, wet soils for incubation and proper embryonic development, but current environmental conditions at high altitudes and latitudes have not restricted their geographic distributions relative to the viviparous Phrynsoma. While oviparous species are able to occur at elevations similar to viviparous species, moisture may become a limiting factor. Timing of parturition coincides with initiation of rains for most viviparous species, however, these species seem able to shift timing of other reproductive events such as mating, ovulation and vitellogenesis to accommodate parturition

during favorable conditions while simultaneously protecting developing embryos from poor environmental conditions. Oviparous species, however, appear limited; all oviparous species exhibit very similar reproductive cycles (Howard, 1974; Pianka and Parker, 1975). Current global warming trends that lead to drying conditions may allow viviparous species to invade new habitats previously limited in distribution by oviparous species. Future invasions of low altitude habitats may be possible by viviparous species with reproductive patterns shifted relative to oviparous congenerics.

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Chapter Three: Status and Prospects of the Texas Horned Lizard (Phrynosoma cornutum): Conservation of a Texas Native*

ABSTRACT

A survey to assess the status of the Texas horned lizard (Phrynosoma cornutum) in Texas was conducted in 1992. Museum specimen records were compiled to assess historical distribution and abundance, and 100 sites across the state with adequate historical records were selected to be surveyed for current presence and abundance of the species. Interviews were conducted with local residents concerning potential correlative factors to horned lizard occurrence, and an extensive database compiled from two sighting surveys distributed statewide was also used for the same purpose. Horned lizards, or evidence of their occurrence, were detected at 48 of the survey sites. These results are discussed in relation to current and historic land-use, pesticide use, and the invasion of the Red Imported Fire Ant (Solenopsis invicta). The future of the Texas horned lizard as a component of the natural heritage of the state is discussed. Phrynosoma cornutum serves as an excellent indicator of the general environmental health of terrestrial ecosystems in Texas. Key words: horned lizards; Phrynosoma cornutum; conservatio*n; fire ants; surveys; Texas.

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INTRODUCTION.

The Texas horned lizard (Phrynosoma cornutum) is a familiar component of the fauna of Texas, and was officially designated the state reptile by the Texas Legislature in 1993. Historically, the species' range included the entire state of Texas except for the easternmost counties, which constitute the Piney Woods (LBJ School of Public Affairs, 1978). These lizards are dietary specialists on harvester ants of the genus Pogonomyrmex (Whitford and Bryant, 1979), which constitute up to 69% of an individual lizard's diet (Pianka and Parker, 1975). P. cornutum adults must utilize several colonies of harvester ants in one day to meet metabolic energy requirements (Munger, 1984). They also feed opportunistically on a variety of other arthropods including grasshoppers, isopods, beetles and beetle larvae (Davis, 1941; Pianka and Parker, 1975; Cohen and Cohen, 1990). Because the species is a specialized insectivore and occurs statewide in a variety of ecotypes, it may serve as an indicator of the general environmental health of terrestrial habitats in Texas: another "canary in the coal mine" (Eldredge, 1991).

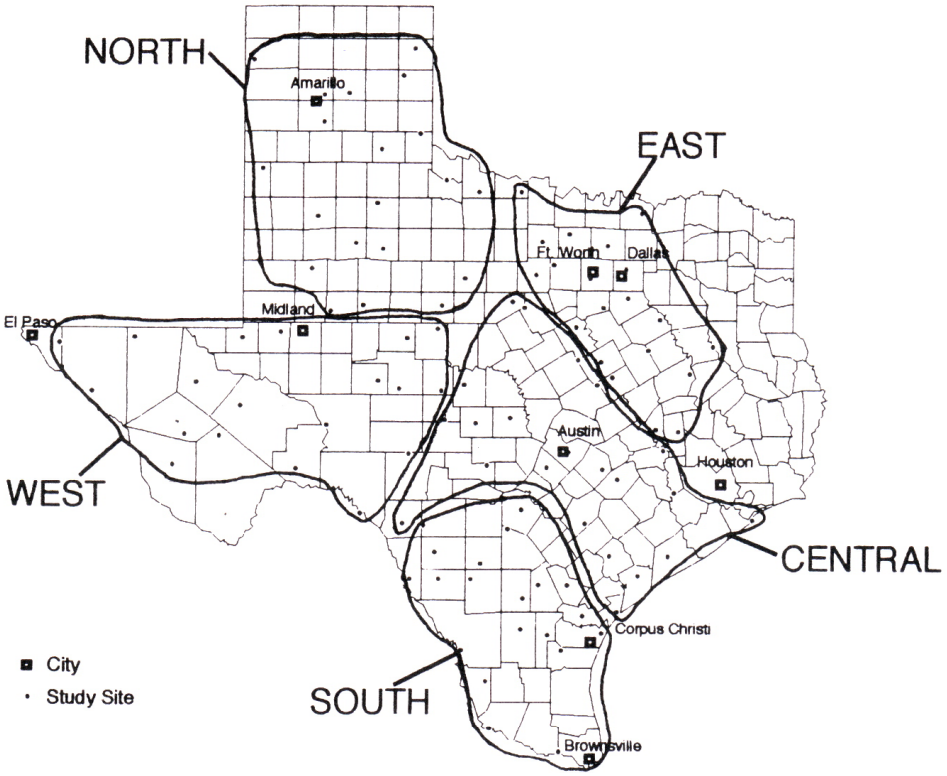
Phrynosoma cornutum is listed as a threatened species in Texas. It was one of the first animals listed by Texas as threatened, on 18 July 1977 (Texas Parks and Wildlife Code, 1987) Ten years prior to the stare's adoption of protective legislation for threatened species, P. cornutum was protected from commercial collection by separate legislation (Bigony, 1981; Welch, 1993; J. Christie, pers. comm.). This protection was originally sought for P. cornutum for reasons including over-collection of specimens for the pet trade, exportation by Boy Scouts who traded them at national jamborees, and utilization in the curio

trade. Recently, concern was focused on the status of Phrynosoma cornutum in Texas (Price, 1990). Anecdotal information indicated P. cornutum had disappeared east of a line extending from Fort Worth to Corpus Christi except for a few isolated and introduced populations, and had become rare and localized in other parts of the state.

Three primary reasons have been suggested for the decline: habitat alteration or destruction by agriculture and urbanization, use of insecticides and other toxic chemicals on crops and to control Solenopsis invicta (the red imported

Figure 20. Areas surveyed for Texas horned lizards in 1992.

Dots indicate specific sites. Outlined regions follow those listed in text.



fire ant), and other direct or indirect effects incurred with the invasion of Solenopsis invicta (Price, 1990). We report here the results of a study designed to assess (1) the historical distribution and abundance of P. cornutum in Texas, (2) the current distribution and abundance of P. cornutum in Texas, and (3) the potential factors implicated in the reported decline of this species.

METHODS

Museum Collection Records

Museum collection records of P. cornutum through 1987 were acquired by surveying institutions listed in Edwards (1975) and others, supplemented with additional records from West Texas State University that included records to 1989. Records from the scientific literature were also collected as well as records from biologists across the state. Ten maps were constructed using Atlas Mapmaker, Version 3.51 showing the number of lizards collected in each county during each decade, with records predating this century included together in one figure (Donaldson et al., 1993).

Sighting Survey

Texans are generally familiar with Phrynosoma cornutum through childhood or current experiences. We considered this collective knowledge to be a valuable untapped resource concerning the status of this species in Texas. Two separate sighting surveys were distributed in an attempt to utilize this resource and obtain broad based information on the current and historical abundance of the species; see Donaldson et al. (1993) for details. Survey A was initiated in the

spring of 1991 when 30 central Texas newspapers, as well as interested individuals, were sent survey forms as a pilot test. In August 1992, Texas Parks and Wildlife Magazine also published the sighting survey (Goin, 1992). In the first section of the survey, the question was asked, "Have you seen a horny toad in the last 10 years?" In the second section, the question "Did you used to see horny toads often?" was asked. Additional information was requested in the event of a positive answer to either question. Thus, individuals could provide information on current and historical sightings of P. cornutum.

Survey B was initiated in the summer of 1992 to accompany the field work (see below). This survey was more limited in scope than the previous survey in that respondents were only queried as to the details of current sightings of P. cornutum. The survey form and a press release were mailed to newspapers published in the counties where field surveys were conducted. Thirty-one of the 197 newspapers that were sent the mailing elected to publish the survey in its original form. In addition, one newspaper published an article on the plight of the Texas horned lizard in which a request was made that sightings of the lizard be reported to us. This article was subsequently picked up by the Associated Press and appeared as an AP wire release in at least five newspapers across Texas. Responses to this article are included in the data analyzed here as they represent a substantial fraction of the responses received in regard to current sightings of P. cornutum.

Data tabulated from survey A included date, time and county of sighting, number and sizes of lizards sighted, comments, and county of origin of the

response. Data tabulated from survey B included date, time, county, exact locality, description of area, weather conditions, number and sizes of lizards, behavior of lizards, and additional comments. County by county statistics regarding numbers of sightings of P. cornutum were compiled. Comments concerning the following categories were noted and tabulated: blood-squirting behavior, interactions with domestic animals, fire ants, harvester ants, land-use, interactions with other wildlife, pesticide use, resurgence in horned lizard populations, and horned lizard population trends. Respondents were not prompted to comment on these particular subjects. In each instance, an effort was made to focus on specific comments in the stated categories. For instance, general comments to the effect that the respondent believed pesticide use had contributed to the decline of horned lizard populations were shunned in favor of specific comments concerning applications of pesticides.

Survey responses were screened to eliminate any possibly erroneous sightings. For instance, sightings of "horned lizards" in excess of 10 inches in length were discounted, as were sightings of "horned lizards" exhibiting uncharacteristic behaviors such as hopping or scurrying up trees. Sightings of horned lizards residing in "shoe boxes" or the like were not included unless the origin of the specimen could be reasonably ascertained. Sightings of dead horned lizards were included.

As the reporting of actual numbers of horned lizards sighted was often imprecise and qualitative, we decided not to attempt to compile those numbers. Instead, a positive response to query 1 or query 2 on survey A was counted as a

single "current sighting" or "historical sighting", respectively, regardless of the number of lizards reported. One such "sighting" was recorded for each county in which the respondent reported seeing or having seen horned lizards. If the number of horned lizards seen and/or other comments by respondents indicated that horned lizards were common in a given county, then the corresponding "sightings" for that county were considered to be "abundant". Responses to survey B were analyzed in a similar manner.

The data from survey A were analyzed as the number of responses originating from counties in each region (#R), the number of current sightings of P. cornutum within each county during the period 1990-1992 (#CS), and the number of historical sightings within each county (#HS). Positive responses to query 1 in which a respondent indicated that their most recent sighting had occurred prior to 1990 were tabulated and are included in a county X decade X sighting matrix and displayed on maps (Table 3, Appendix 2 in Donaldson et al., 1993), but are not included in either #CS or #HS. Values of #CS and #HS can be greater than #R if respondents lived in a different county than where they observed P. cornutum and/or respondents observed P. cornutum in the same county(ies) over several decades.

When the value of at least one of the categories #R, #CS or #HS was 10 or greater within a given county, the following percentage was calculated:

$$\%D = [(\#HS - \#CS) \times 100] / \#HS$$

This percentage is a measure of the relative decline of P. cornutum for each of the five field survey regions.

The following numbers from survey B were tabulated for each county: number of responses originating from that county (#R), number of sightings within that county (#S), and number of sightings within the county categorized as abundant (#A). The percentage of sightings categorized as abundant was also calculated when 10 or more sightings were reported within a given county.

Field Survey

Museum records were used to choose target localities to be surveyed for current presence and abundance of P. cornutum. One hundred localities were chosen on the basis of having a good historical record of the occurrence of P. cornutum, and the specific survey localities matched the historical locations as closely as possible. Exact locations were expanded to include an area within an eight-kilometer radius to insure access to property as close as possible to the historical locations. Localities were also limited to one site per county in an attempt to survey as much of the historical range of the species as possible.

The museum records, unfortunately, exhibit a paucity of localities in the Panhandle due to collection biases. Localities in the Panhandle, therefore, do not have an historical base comparable to the remainder of the state. Localities in the east Texas Piney Woods, such as in Nacogdoches County, were not included because P. cornutum populations there are believed to be introduced (Price, 1990). Alternate sites within the same counties as preferred survey sites were identified in anticipation of problems obtaining access to private property.

Alternate sites were chosen based on the same criteria as preferred sites. Localities were then assigned to central, east, north, south, and west divisions of the state; 20 sites per area (Figure 1) to minimize travel distances. All references to geographic divisions of Texas refer to these designated areas

Field work was conducted between 25 May and 10 October 1992. Site identification numbers, counties surveyed, exact localities, survey dates, expected and observed habitat characteristics, current and historic land-use patterns, pesticide use, and additional information are in Donaldson et al. (1993) or are available from the authors. Field surveys were conducted using time-constrained search techniques (Campbell and Christman, 1982). Five biologists surveyed 20 sites each, and each site was to be surveyed three times between 25 May and 10 October 1992. Permission to access private property was obtained prior to surveying. Data recorded at each site included number of lizards, size (total body length and snout-to-vent length), sex, weight, behavior of individual P. cornutum encountered, photograph of individual lizards captured against color standards, toe-clip identification number, air and soil temperatures at point of capture, time of day, date, weather conditions and location of capture. Clipping was limited to one toe per foot, and clipped toes were retained and preserved in 95% ethanol for future genetic analysis. Additional information about each site was gathered including habitat characteristics, current and historic land-use practices, relative densities of Solenopsis invicta and Pogonomyrmex spp., and soil samples. Photographs of each locality were also taken. Two additional hours were spent at each site interviewing area residents about land-use and pesticide use.

RESULTS

Museum Collection Records

A total of 1,654 museum specimen records, representing 207 (81.5%) of Texas' 254 counties, were compiled for P. cornutum. The earliest record is from Galveston in 1862. These museum records represent a total of 3,262 individual lizards. The largest number of specimens collected was 1,077 from 151 counties in the decade 1960-1969. Of that number, 199 lizards were collected in Tom Green County alone. P. cornutum occurred historically throughout the state except for the far eastern counties, the latter not including counties along the Gulf Coast. P. cornutum does not appear in the far eastern counties prior to 1950, when the species was introduced to places like Nacogdoches. Counties from each designated survey region (Figure 1) are represented in all decades (Donaldson et al., 1993), although North Texas has fewer counties represented. P. cornutum is well represented from Trans-Pecos counties in all maps. South Texas counties, especially near Mexico, also show P. cornutum with a long and continuous history. P. cornutum was collected in Bexar and Travis counties prior to 1900 and up until 1979. It is difficult to observe population fluctuations from museum data because the locations and quantities of P. cornutum collected reflect subjective or opportunistic decisions of individual collectors. At first glance, P. cornutum appears to increase in Texas during the 1950's and 1960's. These decades, however, represent a time period during which W. Frank Blair and his students led an active herpetological program at the University of Texas at Austin, who collected specimens across the state.

Some illustrative data are available, however, for a limited number of localities. For example, 115 P. cornutum were collected in 1969 from the vicinity of Concho Lake, Tom Green County; 18 specimens were collected in one day at College Station, Brazos County, in 1946; 35 were collected east of Lamesa, Dawson County, in 1952; 43 were collected in one month in 1950 east of Stinnet, Hutchinson County; 24 were collected in Lubbock in 1948; 40 and 20 were collected in 1948 and 1960, respectively, from Waco, McLennan County; 14 were collected by one person in one day from Fort Stockton, Pecos County, in 1942; and 26 were collected in Wichita Falls, Wichita County, in 1919.

Sighting Survey

The two surveys have some distinct biases. Whatever biases accrue to the readership of Texas Parks and Wildlife Magazine necessarily accrue to survey A since the overwhelming number of responses came from this group. Most responses to survey B tended to come from smaller metropolitan areas, and we believe this reflects, in part, editorial policies of newspapers to which the survey forms were mailed. Experience has indicated that newspapers in larger metropolitan areas were less willing to publish either of the survey forms in their original format. In addition, the decision of many newspapers in the western section of the state not to print the news release/survey could well be a reflection of the political climate in that region of the state in relation to environmental and land-use issues. Respondents appeared more likely, judging by comments on survey forms, to mail in the survey if they had seen a horned lizard than if they had not seen one.

A total of 700 responses to survey A from 119 different counties were analyzed. Of these respondents, 121 (17%) indicated that they had not seen a horned lizard within the past 10 years while living in Texas, 570 (81%) indicated they had seen a horned lizard during this time, 398 (57%) indicated they had seen one since 1990, and 586 (84%) indicated that they used to see horned lizards often. Current and historical sightings and relative declines in horned lizard abundance by region are summarized in Table 11. A complete county-by-county summary is in Donaldson et al. (1993).

Although the "percent decline" (%D) in sightings should not be taken as an absolute measure of the actual decline in horned lizard populations, it is useful as a relative measure of abundance and trends of sightings between counties or regions. The magnitude of decline represented by sightings appeared to be greatest within counties, which are home to large metropolitan areas. Bexar, Dallas, Harris, Tarrant and Travis counties had percent declines of 76%, 92%, 100%, 85% and 73%, respectively. In contrast, within the less populated counties of DeWitt, Atascosa, Lubbock and Midland (which each had at least 10 responses) the percent declines were 17%, 0%, 0% and 18%, respectively (Donaldson et al., 1993).

A total of 338 respondents reported sightings of P. cornutum in 1992 via survey B. Sightings for each county are given in Donaldson et al. (1993). Results by region are summarized in Table 11. Except in the category of “abundance” results from surveys A and B are combined in the following summary of comments.

Table 11. Summary of sighting survey results.

Individual county tallies are summarized by the regions depicted in Figure 1. (A). Survey A; #R is the number of responses originating in each region, #CS is the number of current (1990-1992) sightings of P. cornutum, #HS is the number of historical sightings of P. cornutum, and %D is a measure of the decline of P. cornutum in each region. (B). Survey B; #R is the number of responses originating in each region, #S is the number of actual sightings in each region, and %A is the proportion of these sightings categorized as abundant.

(A)

Region	#R	#CS	#HS	%D
East	175	64	186	66%
Central	255	114	215	45%
North	48	70	110	36%
South	101	93	128	27%
West	58	83	101	18%
TOTAL	637	424	740	

(B)

Region	#R	#S	%A
East	133	120	28%
Central	72	77	25%
North	48	50	24%
South	49	53	32%
West	35	39	20%
TOTAL	337	339	

Abundance. —One hundred of 570 (18%) sightings of the horned lizard within the past 10 years were categorized as abundant, and 86 of 398 (22%) since 1990 were so categorized. For 1992 sightings (survey B), 90 of 339 (27%) were so categorized.

Domestic animals. —Interactions between horned lizards and domestic animals were commented upon by 15 of 1,038 (1%) respondents. Most of these involved predation by dogs and cats. One respondent witnessed chickens preying upon horned lizards.

Fire ants. —Of the 36 respondents making comments in this category, 13 (36%) reported a decline in numbers of horned lizards following an increase in the population of fire ants, six (17%) noted the presence of horned lizards in their area and the absence of fire ants, and six (17%) observed the simultaneous occurrence of both horned lizards and fire ants. Most of these latter respondents indicated that the influx of fire ants was a recent phenomenon. One respondent from Bee County reported a decline in horned lizards prior to the influx of fire ants. Five other respondents remarked on the presence or absence of fire ants in their area without commenting on any trends in horned lizard populations.

Harvester ants. —Of the 74 responses on this subject, 52 (70%) indicated having observed horned lizards in the presence of harvester ants and 15 (20%) noted a decrease in the abundance of harvester ants prior to or simultaneously with a decline in horned lizards. Most of these attributed the decline in harvester ants to active eradication efforts involving pesticides. Seven (10%) respondents

remarked that harvester ants were present in their area but that horned lizards were not, and two (3%) remarked simply upon the absence of harvester ants.

Land-use. —Comments were received from 26 respondents on this topic; no discernable trend related to horned lizard presence or abundance was apparent. Five (19%) noted a decline in horned lizards when manicured lawns appeared in their neighborhoods, whereas six (23%) noted that horned lizards were locally abundant in yards or vacant lots that were not manicured and retained native grasses. Two respondents (8%) noted a decline in horned lizards associated with the paving of roads. Four (15%) noted declines associated with cultivation of land for commercial crops such as rice, four (15%) noted horned lizards in abundance associated with farm land not currently in production, and three (12%) noted a resurgence of the species on fallow cropland. One person (4%) noted a decline in the Houston area and attributed it to the overall sinking of land, creating a wetter habitat and increasing grass cover. Another respondent (4%) noted horned lizards were abundant on 8.1 hectares of land cultivated to produce guar.

Pesticides. —Fifty respondents in this category noted pesticide/ herbicide use by themselves or other local residents. Ten respondents (20%) said they or their neighbors were actively poisoning harvester ant nests, and five (10%) respondents were attempting to eradicate fire ant nests while simultaneously avoiding harvester ants. Seventeen respondents (34%) noted a decline in horned lizard abundance with increased use of pesticides, and 13 (26%) noted horned lizards doing well in areas where pesticide use was limited or had declined.

Resurgence. —Resurgence of local horned lizard populations within the past few years was attested to by 22 respondents.

Population trends. —Most of the 87 respondents commenting on long-term trends in horned lizard populations thought they had witnessed a significant decline in their area.

Field Surveys

Not all 100 localities originally targeted were completely searched because of weather, problems obtaining landowner permission to survey sites in west and north Texas, and logistical problems. At 17 original localities, property owners would not allow surveying on their property and were hostile towards a surveyor. At one locality in Presidio County, a surveyor was denied access from all property owners within eight kilometers of the site, areas surrounding it, and at an alternate locality. This survey ultimately had to be done on county property. At another site in East Texas, a surveyor was not allowed to resurvey after the initial visit because the landowner was subsequently told by friends to be cautious about letting anyone on his property looking for protected species; remaining visits were done on surrounding property within the eight kilometer radius specified in the methods. A total of 78 localities were surveyed all three times, six were surveyed twice, 13 were surveyed once, one locality was surveyed once for only two hours, two localities were visited but not surveyed, and one locality was not visited at all. All 20 localities in Central, East and South Texas and nine localities in both North and West Texas were completely surveyed.

Phrynosoma cornutum encountered during the survey. —

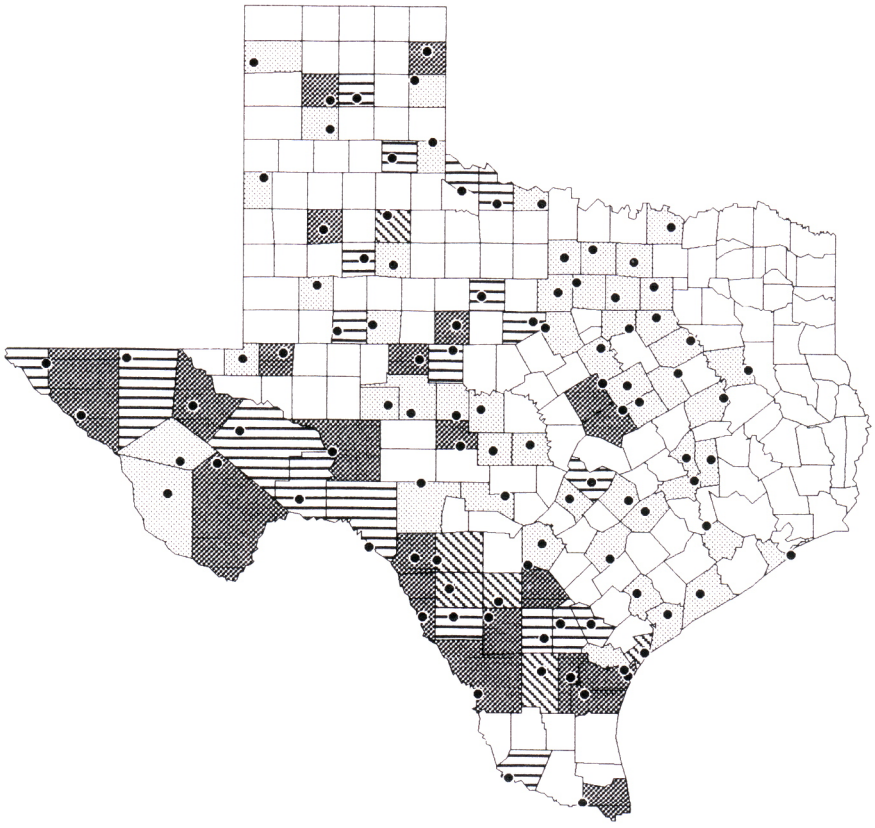
A total of 145 individual Phrynosoma cornutum were encountered during field surveys, including lizards, which were captured and marked, found dead, and escaped capture (Figure 21). These included 46 females, 41 males, and 41 hatchlings and juveniles (escapees and some dead lizards were not sexed). The majority of lizards were found between 8 June and 24 August 1992. Encounters confirm that daily lizard activity during summer months is bimodal. Lizards were first encountered in a variety of situations: 20 on dirt and paved roads, 32 under vegetation (grasses, shrubs, trees), 17 on grass clumps or mowed grass, 14 on bare soil, three in vegetable gardens, one stuck in a vine-covered piece of chickenwire fence, and 23 on open ground running into thick vegetation. Nineteen lizards escaped capture. Of the 19 dead P. cornutum, 12 were found on roads or roadsides and presumably were killed by vehicular traffic. The cause of death for the seven remaining is unknown, but is suspected to be natural predation in at least two instances.

Ninety-nine P. cornutum were captured and marked at 26 localities: two in Central Texas, ten in South Texas, eight in North Texas, and six in West Texas. Only two lizards were seen driving between locations, and only four were recaptured. One lizard was recaptured twice and one was recaptured on the day it was marked while the surveyor was walking to a new area on the survey site.

Most P. cornutum were found in South Texas where 65 were marked (Donaldson et al., 1993). Fifty-six percent (55/99) of the marked P. cornutum were found at five localities: 19 in Zavala County, 12 in Frio County, ten in

Figure 21. Field Survey Results.

Texas map showing results of the field survey for Texas horned lizards. Dots indicate survey sites. Counties indicated by light gray stippling contain 52 sites where no evidence of P. cornutum was found. The counties indicated by dark gray stippling contain 22 sites where only indirect evidence (sighting reports by local residents, scat, or dead lizards) of P. cornutum was found. Horizontal lines indicate counties containing the 20 sites where 1-4 P. cornutum were captured and marked. Slanted lines indicate counties where 5-19 lizards were marked.



Aransas County, and seven each in Duval and Uvalde counties. No localities in other regions of the state had as many P. cornutum marked, but two were comparable. Six lizards were marked at a site in North Texas (Dickens County), and five lizards were marked, two were found dead, and one escaped at a site in Central Texas (Eastland County).

An additional 22 localities, four each in Central and North Texas, eight in South Texas, and six in West Texas, provided evidence that P. cornutum existed, but live lizards were not encountered (Donaldson et al., 1993). Such evidence included fecal samples (scat), dead individuals, and landowners' sightings. Neither live P. cornutum nor evidence of the species' occurrence was found at the remaining 52 sites.

Relative abundance of Solenopsis invicta and Pogonomyrmex spp.—

The size of surveyed areas varied according to habitat conditions present at each site; estimated sizes ranged from 0.83 hectares to 38 hectares. Relative abundance of Solenopsis invicta and Pogonomyrmex spp. were calculated based on the estimated area surveyed and number of mounds counted at each site. In East Texas, density of S. invicta was so high that the surveyor opted to record number of mounds encountered per meter along a transect rather than enumerate every mound: at four sites, fire ant mounds occurred every one to three meters. In central Texas, density of fire ant mounds ranged from 0/ha (five sites) to 671/ha (one site). No fire ants were reported from nine sites completely surveyed in North Texas, and no fire ants were reported from West Texas sites. S. invicta mound densities in South Texas ranged from 0/ha (12 sites) to 281/ha (one site). S. invicta was present at only five of 30 localities where individual P. cornutum were marked, found dead, or escaped capture. Densities of S. invicta mounds changed very little throughout the summer. Their activity was considerably reduced in mid and late summer when the ground dried and it was very hot. More

time was required to check mounds for activity because the ants were deep underground.

Densities of Pogonomyrmex spp. were also estimated based on the number of nests encountered in each survey area. These estimates are conservative because, in many cases, surveyors would find wandering foragers but could not locate the nest entrance. This may seem unusual since harvester ants typically clear all vegetation and debris from the nest entrance to form a characteristic circle, but in several cases nests were found without any clearing at all; only a hole in the ground was observed. Alates (winged sexuals) were observed throughout the summer on several sites in Central Texas. Densities varied in all areas. In Central Texas, nest densities ranged from 0/ha (three sites) to 24/ ha. In East Texas, nest densities ranged from 0/ha to one every three to six meters. In North Texas, nest densities ranged from 7/ha to 26/ha.

In South Texas, nest densities ranged from 0/ha (one site) to 38/ha (one site), and in West Texas from 0/ha (nine sites) to 25/ha. Only three of 30 sites that had positive evidence of P. cornutum lacked Pogonomyrmex and all three were in West Texas. Maximum density of harvester ant nests on sites with horned lizards was 38/ha. Pogonomyrmex nest densities changed at several locations during the course of the survey. At one location in East Texas, four nests were found during the last visit that had not existed during prior visits. At two locations in Central Texas, six nests were taken over by Solenopsis invicta. Evidence of displacement included dead bodies of harvester ants and the presence of S. invicta in the upper seed chamber of harvester ants' nests.

Resident/Property Owner interviews: pesticide use.—

Interviews were conducted at 97 localities during the survey. Many persons were uneasy when asked questions about their property, especially questions about pesticide use. Three landowners refused to answer pesticide use questions entirely. Historic pesticide use is ambiguous in many cases because residents or property owners had been on the property for only 20 to 25 years, and in some cases for even shorter periods (one to five years.)

Currently, pesticide use is widespread throughout Texas. Pesticide use on or near the property was reported at 76 of 97 (78%) survey sites. Only seven sites (7%) reported no pesticide use, and at 14 sites pesticide use was unknown. Residents at 36 of the 97 localities (37%) used pesticides specifically to kill harvester ants. Pesticides and other chemicals used on insects, crops, and "weeds" included Amdro, Diazinon, diesel fuel, gasoline, Greenlight Fire Ant Killer, Orthene, Logic, Ortho Pest Granules, Spectracide, AG500, Round-up, Mirex, Malathion, Scourge in mineral oil, Resmethrin, TAT ant traps, Dursban, Sodium silicate in baby powder, Thimet, Ridomil, Sett, Pydrin, Lorsban, Pix, Arsenic dust, 2-4D, Methyl bromide, Sevin dust and liquid, Ortho Flying Insect Spray, KGRO Fire Ant Killer, Paraquat, Valpar, Benylate, Aggie Ant Killer, Treflan, Typersan, Daconil, and Prowl. Pesticide application methods varied and included hand application on single ant mounds, dusting entire yards or pastures, spraying small or large areas using tractors, and aerial applications by crop dusters.

Of the 30 sites where P. cornutum were marked, escaped or found dead, 22 landowners (73%) used pesticides on their property, 11 (37%) used pesticides

on Pogonomyrmex spp. nests, and three (10%) reported no pesticide use. Current pesticide use at this subset of 30 sites was not appreciably different from the total set of study sites.

Pesticides used in the past included DDT, calcium arsenate, chlordane, and Snake Away. Information about past use is limited because many people could not remember what had been used. Individual counties have been spraying roadsides to control mosquito outbreaks for at least 25 years in some areas, such as along the Gulf Coast. One recurrent comment was that cotton crops require the use of a wide array of chemicals. Chemical use on cotton began in the 1930's with calcium arsenate, but intense use of chemicals did not begin until after World War II. It is during this time that chemicals began being used on a large number of other crops as well (Metcalf, 1980).

Resident/Property Owner interviews: land-use.—

Land-use in Texas is highly variable, and current land-use patterns do not necessarily reflect historic land-use. Many residents interviewed had not lived on their property for longer than 20 or 25 years, making it difficult to gain a historical perspective on land-use. Nevertheless, abundant historical information within the last two decades as well as current information on land-use was obtained (Donaldson et al., 1993).

Land-use at 13 of the 26 sites where P. cornutum was captured and marked consisted primarily of ranch land for grazing cattle. These sites had also been used historically for ranching for 30-100 years. Some ranches have limited areas of agricultural crops surrounding them. Small parcels on ranches are planted

in corn, oats, or hay for livestock to eat during the winter. Of the remaining sites, three were state parks and one was a National Park. Six sites were in small communities or residential areas bordered by ranch land. One site had been a vegetable farm since 1948 with minimal pesticide use reported and crops were hand-picked. One site was a county airport that rarely received aircraft, and land-use at the remaining site is unknown because the information was not recorded during the single visit.

Land-use at 22 sites where only evidence of P. cornutum was found included ranching, small communities, state parks and wildlife management areas (WMA), and a resort community. Eleven sites were ranches, two of which had been cropland until 1980, four were surrounded by cropland, and one was heavily grazed. The ranches had existed for 12 to 40+ years. Three sites were small communities, one of which was cropland approximately 30 years ago and one of which was surrounded by cropland. Three sites were state parks and one a WMA. The WMA was surrounded by cropland, and one state park was situated where increasing condominium development had taken place during the last five years. One site was a resort community that left the back section undeveloped except for a maze of dirt roads and cut and cleared swaths.

Land-use at sites where no evidence of P. cornutum was found is more variable. Twelve sites were residential/urban areas. Three sites were small residences with pastures, which had existed 7-25 years. Two sites were adjacent to county airports, one of which was also used to produce hay twice a year. Six

sites were state parks and recreational areas classified as high-use by the state park system.

DISCUSSION

Populations

Phrynosoma cornutum populations appear to be robust in South Texas, where individuals were captured and marked at 10 localities, and evidence of the presence of P. cornutum was found at an additional eight localities. Texas horned lizards also appear to be doing well in North and West Texas; but, the inability to completely survey some sites in both areas render results ambiguous. West Texas, in particular, should have produced better results, since the species is historically common and none of the putative factors influencing populations of this species are known to be operating there. Field surveys confirm public perception and historical data that P. cornutum has declined in East and Central Texas. The greatest decline of P. cornutum has occurred in East Texas where no individuals were found. Central Texas also shows an apparent loss of populations; only six sites show positive evidence that lizards were still there, and lizards were actually captured at only two of these sites. At the remaining four sites, property owners had seen lizards only rarely or only scat were found.

Pesticides

Pesticide use does not seem to be a strong factor influencing the presence or absence of P. cornutum since the pattern of pesticide use on sites with positive evidence of the lizard was similar to that for all sites surveyed. Pesticide use

information is qualitative, however, and the assumption that pesticides play no part in declining P. cornutum populations should be viewed with caution. Results show that 37% of property owners are currently using pesticides to kill Pogonomyrmex; since the Texas horned lizard specializes on these ants for food, eliminating them will have a negative effect on populations in the future. We do not know how P. cornutum may be affected if individual lizards eat poisoned ants. Hibernating or aestivating lizards and incubating eggs may also be susceptible to applied chemicals that leach through the soil. Pesticide use did not become widespread in Texas until the late 1940's-early 1950's, and pesticides are used in larger quantities on cropland than land with other uses.

Land-use

In the scope of this study, land-use is the primary indicator for the presence or absence of P. cornutum. Agriculture seems to be the primary factor associated with the absence of P. cornutum populations on the study sites, with urbanization running a close second. Agricultural activities can directly lead to declining populations through several different avenues. Since horned lizards hibernate or aestivate at soil depths routinely disturbed by plowing or tilling, such activity may kill them directly or indirectly by exposing lizards to harsh climatic conditions they seek to avoid. If plowing occurs in the summer, incubating P. cornutum eggs could be destroyed. Plowing land also destroys the habitat of P. cornutum, forcing lizards into surrounding, often suboptimal habitats as witnessed by several property owners questioned during this survey. Crops tend to promote the use of chemicals in the area. Several persons interviewed said that P.

cornutum was abundant around and in cotton fields in the 1930's and 1940's, when pesticide use was low and cotton was hand-picked instead of being harvested by using defoliants and machines.

Fire ants

Impact of Solenopsis invicta on P. cornutum is unclear. Scale effects may be important as demonstrated for small mammals (Killion and Grant, 1993). Many areas where this ant species occurs in high densities are also areas subject to landscape disturbances (Porter and Savignano, 1990). Five instances of P. cornutum swarmed by ants were recorded, but the lizards were already dead, and their deaths cannot be directly linked to fire ants. S. invicta may impact P. cornutum in a variety of ways, however. It is unknown whether horned lizards can forage, grow and reproduce normally on a diet consisting of S. invicta when other ant species become rare. WLH has had experience trying to rear hatchling P. cornutum on a diet of fire ants and was unsuccessful, the lizards refused to eat the ants after a short period of time (unpublished data). Evidence that fire ants kill other arthropods including Pogonomyrmex spp. was recorded during this status survey, and their negative impacts on the diversity and abundance of native arthropods have been well documented (Porter and Savignano, 1990; Morris and Steigman, 1993). Such impacts may decrease availability of adequate food resources to the Texas horned lizard. Another feature of S. invicta, which may affect P. cornutum, is the subterranean foraging tunnel system these ants construct below their mounds (Markin et al., 1975). In areas of high S. invicta densities, it

may be impossible for horned lizard eggs to incubate or individuals to hibernate successfully.

PROJECTED OUTLOOK FOR PHRYNOSOMA CORNUTUM IN TEXAS

Populations of Phrynosoma cornutum in South and far West Texas will probably remain stable unless landscape-scale changes occur in land use. Solenopsis invicta is not expected to invade most of South Texas because of the hot, dry climate, but it probably will invade urban and residential areas as it has in West Texas (Drees and Vinson, 1991). We suspect the remaining disjunct populations in East and Central Texas will continue to decline with continued agricultural activities and urbanization, with the ongoing invasion of Solenopsis invicta a contributing factor. Populations in North Texas may also decline in areas where these factors operate.

Conservation measures to restore Phrynosoma cornutum to areas where the species has declined or disappeared should address reestablishment of native vegetation communities, and maintenance of vegetation corridors that remain unplowed and untreated with broadcast chemicals. Domestic animals such as dogs and cats should be controlled to reduce predation pressure. Conservation measures should seek to prevent invasion of Solenopsis invicta, or to control this species while minimizing impacts to native arthropod communities. It should perhaps go without saying that commercial trade in this species should be curtailed and existing regulations to that effect enforced.

ACKNOWLEDGMENTS

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Chapter Four: The Next Step - Dynamic Visualization Tools for Implementation of Phylogenetic Hypotheses and Reconstruction of Ancestral States Using Three-dimensional Data.

(A collaborative effort between Timothy B. Rowe, University of Texas at Austin, and Theodore Garland, Jr., University of California at Riverside.)

Upon completion of a doctoral degree at the University of Texas at Austin, I will begin a National Science Foundation Postdoctoral Research Fellowship in Biological Informatics that combines elements in my research and takes it to another step. I will build on the phylogeny created in Chapter 1 and begin work resolving the phylogenetic relationships of Phrynosoma using nuclear genes. After refining the phylogeny, techniques used in Chapter 2 on reconstruction of ancestral states will be used to combine mathematical algorithms with morphing programs that take images of end state taxa to recreate ancestral images. Image data will be high-resolution x-ray computed tomography scans of lizards. These scans are comprised of millions of facets – a large amount of data. Tools and skills developed in Chapter 3 to handle large data sets will be used to help manage data for this project. The following is a description of the research program outlined and submitted to the National Science Foundation.

ABSTRACT

Reconstructing historical relationships of diverse species is a basic goal of systematic biology and essential to comparative biology. Species represent terminal points in evolutionary time and are not independently distributed. Mathematical and statistical methods are available to sort through phylogenetic correlation among species, and this information can be used to reveal concealed evolutionary patterns. Computational tools will be developed to model horn evolution in a genus of lizards, Phrynosoma. Three-dimensional data generated by high-resolution x-ray computed tomography (CT) will be combined with phylogenetic hypotheses and algorithms to compute ancestral horn states. The primary hypothesis, horn number increases throughout the phylogenetic tree from ancestor to terminal taxa, will be tested. Additional objectives of this research are to create visualization tools that 1) reconstruct horn morphology in ancestral Phrynosoma from extant species and 2) dynamically show changes in horn characteristics from ancestor to extant species in three-dimensions. These objectives will be met by incorporating results from phylogenetic analyses of molecular data with mathematical and statistical techniques for ancestral trait reconstruction applied to three-dimensional CT data. Application of 3-D morphing to systematics and comparative methods to reconstruct ancestors is a novel approach in visualization methods. All CT data will be available through a NSF Digital Library.

INTRODUCTION

Reconstructing historical relationships of diverse taxa is a basic goal of systematic biology and essential to comparative biology. Before molecular data became available, morphological characteristics were predominantly used in systematics. Development of widely used and accurate molecular techniques has lead to a surge in available data for systematists to use in reconstructing evolutionary relationships among diverse taxonomic groups in the form of phylogenies (Hilllis, et al 1996). Phylogenies are tools comparative biologists use for understanding evolution of complex phenotypes and behaviors. Extant taxa represent terminal points in evolutionary time and are not independently distributed; closely related taxa are more likely to be similar to each other than distantly related species (Felsenstein, 1985). The extent to which species resemble each other based on historical relationships must be considered in any comparative study.

Several mathematical and statistical methods are available to sort through phylogenetic correlation among species, and this information can be used to reveal concealed evolutionary patterns (Felsenstein, 1985; Garland, et al, 1992, 1993, 1999; Martins and Hansen, 1997; Pagel, 1998; Garland and Ives, 2000; Lapointe and Garland, 2001). Models of how different systems evolve can be examined throughout a range of natural groups and reveal general or novel evolutionary patterns, rates of change among different lineages, and where in lineages particular phenotypes or behaviors evolved (Brooks and McLennan, 1991; Eggleton and Vane-Wright, 1994; Funk and Brooks, 1990; Garland, 1992;

Garland, et al, 1997; Harvey and Pagel, 1991; Martins, 1996; Wainwright and Reilly, 1994). Growing use of phylogenetic information has led to a rapid increase in methods for accurate ancestral reconstruction estimates (Cunningham, et al, 1998; Garland, et al, 1997; Maddison, 1995; Oakley and Cunningham, 2000; Omland, 1999; Polly, 2001; Schultz and Churchill, 1999). Ancestral state reconstruction has generated innovative and creative research in biology to understand underlying conditions that have generated the rich diversity of species on earth (Iviks, et al, 1997; Jermann, et al, 1995; Ryan and Rand, 1995, 1999; Schluter, et al, 1997).

This project will add to a rapidly developing field by integrating new visualization tools with methods to reconstruct ancestral traits. These tools will allow scientists to peer into the past, to see what intermediate or ancestral states look like, and to visualize changes as they occur through branches in evolutionary relationships. Using three-dimensional data generated by high-resolution x-ray computed tomography, combined with phylogenetic hypotheses and algorithms to compute ancestral states, tools will be developed to model ancestral taxa and dynamically visualize changes from ancestor to extant species. A genus of lizards, Phrynosoma, will be used to model horn evolution. Phrynosoma species (horned lizards) exhibit diverse and complex morphological characters, yet remain a tractable group with 13 extant species that are biologically interesting and have widespread public appeal. They share a general skull feature, a crown of horns, yet this feature is used to diagnose and distinguish each species. The primary hypothesis, horn number increases throughout the phylogenetic tree from ancestor

to terminal taxa will be tested. Many extant and extinct taxa exhibit amazing varieties of skull ornamentation, and a general model of horn evolution in Phrynosoma may help explain evolution of general ornamentation throughout the animal kingdom.

OBJECTIVES, METHODS, AND SIGNIFICANCE OF RESEARCH

Objectives of this research are to create visualization tools that 1) reconstruct horn morphology in ancestral Phrynosoma from extant species and 2) dynamically show changes in horn characteristics from ancestor to extant species in three-dimensions. These objectives will be met by incorporating results from phylogenetic analyses of molecular data with mathematical and statistical techniques for ancestral trait reconstruction applied to three-dimensional (3-D) morphological data generated from a high-resolution computed tomography (CT) scanner. CT scanning has been available for medical purposes since the 1970s, but it is an underutilized resource in other biological fields. Volumetric, three-dimensional visualization programs are used for treating complex problems in medicine (Taylor, 2000). This project uses 3-D data generated from CT in an entirely new realm of collaborative learning and discovery. Software will be developed to integrate 3-D data with phylogenetic and comparative analyses to create images of hypothesized ancestors using a technique called "morphing" (an abbreviated term for metamorphosing). Morphing is a dynamic process that allows visualization of transformational changes from one form to another in real time (DeCarlo and Gallier, 1996). Application of 3-D morphing to systematics and comparative methods to reconstruct ancestors is a novel approach in

visualization methods. These tools will not only be important for understanding evolution of complex traits such as horn evolution, but they will enable physicians to see changes in tissues related to disease, and they will give molecular biologists tools for visualizing changes in protein conformation.

Computed Tomography Data

Traditional morphological approaches in systematics and comparative studies generally score discrete or continuously varying characters as data for analyses. These data are taken from external or internal characters; however, techniques for gathering data on internal characters formerly required destruction of specimens in part or whole. This project will use 3-D images obtained by scanning biological specimens with high-resolution x-ray computed Tomography. CT scanners were originally developed for the medical field, but their utility is just now being realized in other fields. CT scanning allows visualization of features in the interior of solid objects and for obtaining information on their 3-D geometry without destroying a specimen (Conroy and Vannier, 1984). X-rays are passed through a specific plane of an object rotating on a turntable and recorded by an array of detectors. Detectors record information on attenuation of x-rays passing through an illuminated plane at various angles and at various times during rotation. A resulting file can be interpreted as a two-dimensional image comprised of several thousand points in a plane or slice, recording a density map of the sample. By sequentially imaging many two dimensional slices of a specimen, its structure is assembled into a 3-D image (Figure 22). Two species of horned

lizards, P. cornutum and P. taurus, have been scanned at the University of Texas at Austin

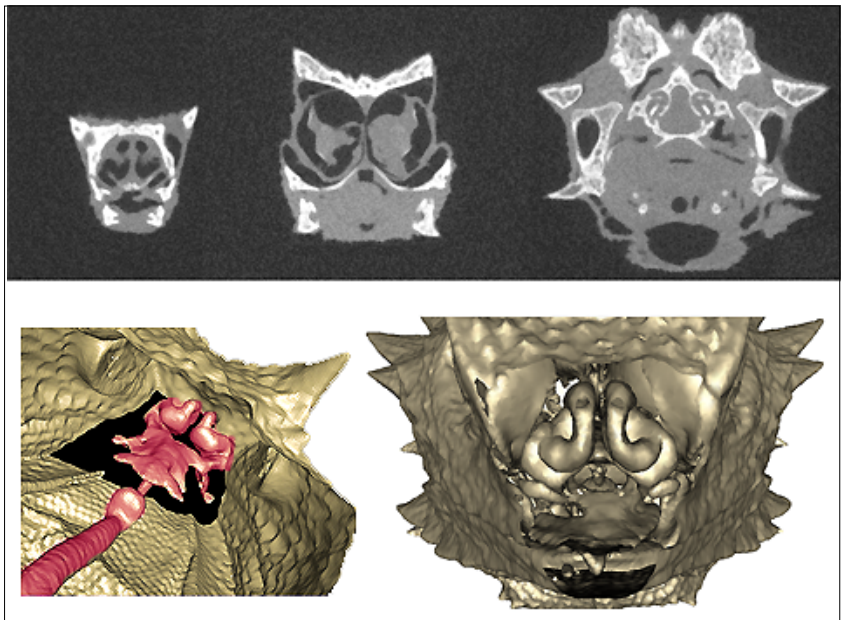


Figure 22: Image files created from high resolu
Row: 3 coronal slices of P. cornutum head. Bottom Row: I
P. taurus internal view of head shown as an isc
density value set for epithelial tissue. Right
cornutum is an isosurface rendering and cut awa
internal structures of the head.

High-Resolution X-ray CT Facility to explore data generated by CT. This facility is designated as a NSF-supported shared multi-user facility and is the only high-resolution facility in academia. Images and movies generated from CT scanning P. taurus and P. cornutum are available at:

http://www.ctlab.geo.utexas.edu/dmg/clado/projects/lepidosaurs/cornutum/index.html

http://www.ctlab.geo.utexas.edu/dmg/clado/projects/lepidosaurs/Ptaurus/index.html

http://www.tacc.utexas.edu/~reyes/hornytoad/hornytoad_heads.html

Before image analysis, several verification steps and corrections must be performed to correct for distortions and x-ray drift during scanning. A histogram of each slice is plotted separately, and a composite histogram of all slices is made to verify that images are within the allowable 16-bit density range of 0 to 65536. Various peaks in the histogram represent values of air, tissue, and bone. Minimum peak values correspond to air densities and are useful for determining x-ray drift between successive slices. A filter can be used to determine the average peak value, and each file can be corrected to this value. In addition to drift correction, noise is also present, and a 3x3 median filter is applied to reduce noise and provide cleaner images.

Programs like NIH-Image, ImageJ, and Visualization Tool Kit (VTK) can be used to convert 2-dimensional, corrected, slices into 3-D models (Figures 1 and 2). VTK produces a 3-D image as an isosurface based on specified density values. Density values from histograms produced in earlier steps are used to specify values in VTK to create images of different tissues. Triangulated images can also be projected from CT data. Triangulated images contain over 700,000 facets in lizard skulls alone and can be used to select control points with X, Y, and Z coordinates or to select groups of facets corresponding to homologous features. Instead of using single measured traits (e.g. femur length, orbit diameter), whole features can be selected (e.g. temporal horns) and a matrix of specified values will represent traits being compared. Selection of whole 3-D features from CT data reduces subjectivity and measurement error (Conroy and Vannier, 1984). New data can also be analyzed (e.g. endocasts of brain cases) because these data were

not previously available without destroying specimens. High performance computers are necessary to open, read and manipulate data files that can be over 100 megabytes in a single triangulated image. CT scans of the remaining eleven Phrynosoma species will be obtained during this fellowship at the University of Texas at Austin High-Resolution X-ray CT Facility, and images and data generated from scans will be submitted to NSF Digital Libraries.

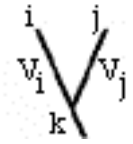
Phylogenetic Analysis and Ancestral Reconstruction

Molecular phylogenetic analysis of a genus of lizards, Phrynosoma, is part of my Ph.D. dissertation research and only a brief description of the analysis will be given with the understanding that this analysis is not part of the postdoctoral fellowship. Phrynosoma is a group of 13 recognized species of lizards that occur from southern Canada to Guatemala. Species are morphologically distinct and form a recognizable monophyletic group within Phrynosomatidae (Frost and Etheridge, 1989; Reeder and Wiens, 1996). Outgroup taxa for horned lizards do not have horns on their heads, but each Phrynosoma has its own unique horn ornamentation. This genus is ideal for this project because it exhibits complex variation in horn structure, yet is a manageable size. Analyses of interspecific relationships of horned lizards were conducted based on four mitochondrial gene sequences: cytochrome *b*, ND4, 12S RNA, and 16S RNA. Phylogenetic hypothesis from this analysis forms the foundation for ancestral reconstruction and will be used as the evolutionary model of branching relationships and branch length estimates in this study. Additional resolution of basal relationships within the genus will be sought prior to beginning the postdoctoral work.

Because species are related to one another hierarchically, they cannot be regarded as independent for statistical purposes (Felsenstein, 1985). Mathematical models attempt to correct or account for phylogenetic correlation between species in comparative studies by using information about species relationships contained in phylogenetic trees (Felsenstein, 1985; Garland, et al, 1993; Martins and Hansen, 1996, 1997; Purvis and Garland, 1993; Garland and Ives, 2000; Lapointe and Garland, 2001). Models used in comparative studies calculate internal nodes of phylogenetic trees that represent presumed ancestors of extant, terminal taxa.

Two straightforward methods will be used to reconstruct ancestral states for this study: independent contrasts (Felsenstein, 1985) and a general linear model (Martins and Hansen, 1997; Garland, et al, 1999). Independent contrasts are calculated as follows:

$$X_k = \frac{v_j X_i + v_i X_j}{v_i + v_j}$$



Where X is a phenotype and v is the expected variance of evolutionary change in terms of branch lengths, i and j are terminal species and k is a common ancestor (image on right). Martins and Hansen's (1997) general linear model is equally straightforward:

$$Y = X\beta + \varepsilon$$

Y is a vector of ancestral character states, X is a vector of extant character states, β is a matrix describing similarities between Y and X due to phylogenetic correlation, and ε is a vector of error terms. Both methods require knowing the species' branching pattern and branch lengths, which are generated by most

programs used in systematic studies. These equations and associated parameters will be incorporated into morphing software to generate visual images of estimated ancestral characters. The ultimate goal will be the visualization of the ancestor of all horned lizards to map the evolution of horns in the group and support or reject the hypothesis that horn number increases throughout the phylogenetic tree.

Morphing

Morphing programs are available for transforming two- and 3-D objects into each other using linear equations. Two objects with corresponding control points are designated; one object serves as a starting state and a second object is the end state. The morphing process then transforms one object into another using directed line segments connecting equal numbers of corresponding control points. For regions of an image not covered by line segments, a weighted average of features is used (Beier and Neely, 1992; Lerios, et al, 1995). Problems to overcome in this proposal are 1) the objects being morphed are highly complex 3-D objects with hundreds of thousands facets and surfaces which do not correspond in number between two end states and 2) the morphing process will be used to reconstruct hypothetical ancestral states from terminal states rather than transforming designated states into each other. Characters for morphing will be chosen from triangulated polygonal reconstructions from CT data. Characters differ in topology between species (Figure 23). Transformations between two different characters involve changes in surface topology, which describes the shape of a character specified by the connectivity of the surface in terms of a

mesh (DeCarlo and Gallier, 1996). A transition between two surface meshes with different topology can be produced by introducing duplicate or degenerate surface mesh elements interpolated using a deformation approach derived from a data fitting process (Bethel and Uselton, 1989; Delingette, et al, 1993). Though topologies in two shapes can differ, the number of corresponding control points between two shapes must be equal otherwise surfaces split open during the morphing process. In graphics applications, the problem has been averted by identifying and constraining changes based on intermediate shapes that have matched "safe" geometries where areas at risk of opening are collapsed using control curves (DeCarlo and Gallier, 1996). However, in the present study, reconstruction of intermediate steps is the goal.

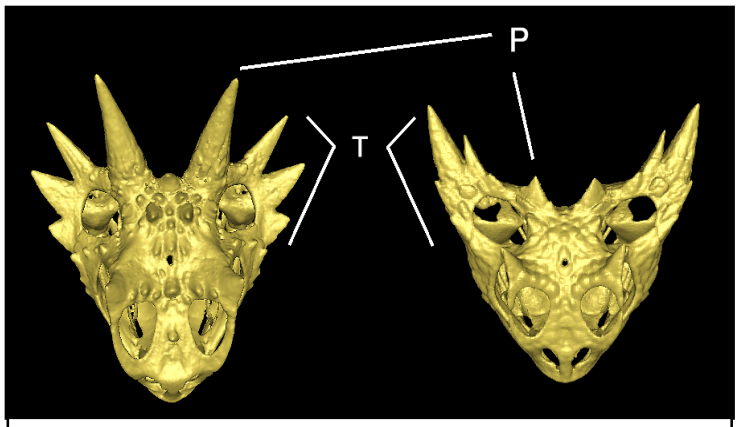


Figure 23: 3-D renderings of P. (torosus) and T. (triceratops) taurus (R) showing differences in T (temporal horns) and P (parietal horns).

A solution to morphing objects with unequal control points is to use data fitting algorithms to match control points and add points in objects' surface meshes. Other techniques will be investigated that will provide more user controlled approaches where the user identifies homologous regions of a topology and control points are equilibrated on a case by case topology. Topological

transitions will be modeled using equations discussed above in reconstruction of ancestral traits.

CO-SPONSORSHIP AND COLLABORATION

The NSF postdoctoral fellowship in biological informatics gives me the opportunity to build on skills and knowledge acquired during my doctoral education and form new collaborative relationships between computer scientists, biologists, and paleontologists at the University of California at Riverside and the University of Texas at Austin. The realization of this project will require the efforts of several unique resources available at both institutions and will provide a strong basis for future shared programs between two diverse universities and their departments. This project will add to a rapidly developing field in biological informatics. It represents unique and creative combinations of state-of-the-art technology and mathematical approaches to apply to basic scientific questions, creating new tools for many disciplines.

Tools developed in the course of this research program will allow scientists to peer into the past, to see what intermediate or ancestral states look like, and to visualize changes as they occur through branches in evolutionary relationships. These tools will not only be important for understanding evolution of complex traits such as horn evolution, but they will enable physicians to see changes in tissues related to disease, and they will give molecular biologists tools for visualizing changes in protein conformation. All products from the CT facility will be available to the public through the NSF Digital Library. All programs and tools derived from this research will be available in the public domain.

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Appendices

APPENDIX 1: SPECIMENS AND MATERIALS EXAMINED

Tissues and DNA for genetic analyses were obtained from the following specimens. GenBank accesssion numbers are on the following page.

Species	Number Identification	Specimen Voucher
Callisaurus draconoides	1	LSUMZ 48811
Cophosaurus texanus	2	LSUMZ 48758
Holbrookia maculata	3	LSUMZ 48805
Phrynosoma asio	4	RRM 2499
Phrynosoma braconnieri	5	WLH 01111
Phrynosoma cornutum	6	LSUMZ 48807
Phrynosoma coronatum	7	RRM 2479
Phrynosoma ditmarsi	8	RRM 2459
Phrynosoma douglasii	9	BJ 961/t011
Phrynosoma hernandesi	10	RRM 2470
Phrynosoma mcallii	11	ROM 13876/WLH 10059
Phrynosoma modestum	12	LSUMZ 48831
Phrynosoma orbiculare	13	RRM 2480
Phrynosoma platyrhinos	14	ASU 15250
Phrynosoma solare	15	ROM 15044
Phrynosoma taurus	16	NA*
Sceloporus merriami	17	LSUMZ 48844
Urosaurus ornatus	18	LSUMZ 48828
Uta stansburiana	19	LSUMZ 48840

* See Reeder and Montanucci (2001)
ASU: Appalachian State university
LSUMZ: Louisiana State University
RRM: Richard R. Montanucci
ROM: Royal Ontario Museum (Robert W. Murphy)
WLH: Wendy L. Hodges

GenBank Accession Number					
Number Identification	**12S rRNA**	**16S rRNA**	**Cytochrome *b***	**ND4**	**tRNA-His**
1	L40437	L41441	AY141099	AY141061	AY141080
2	L40438	L41442	AY141100	AY141062	AY141081
3	L40440	L41445	AY141101	AY141063	AY141082
4	L40446	L41452	AY141086	AY141048	AY141067
5	NA	NA	AY141098	AY141060	AY141079
6	L40447	L41453	AY141087	AY141049	AY141068
7	AF346839	AF346846	AY141088	AY141050	AY141069
8	AF346845	AF346852	AY141089	AY141051	AY141070
9	NA	NA	AY141090	AY141052	AY141071
10	L40448	L41454	AY141091	AY141053	AY141072
11	AF346840	AF346847	AY141092	AY141054	AY141073
12	L41455	L40449	AY141093	AY141055	AY141074
13	AF346841	AF346848	AY141094	AY141056	AY141075
14	AF346842	AF346849	AY141095	AY141057	AY141076
15	AF346843	AF346850	AY141096	AY141058	AY141077
16	AF346844	AF346851	AY141097	AY141059	AY141078
17	L41418	L41468	AY141102	AY141064	AY141083
18	L41436	L41487	AY141103	AY141065	AY141084
19	L41438	L41489	AY141104	AY141066	AY141085

APPENDIX 2: DATA MATRIX

The data matrix used in analyses is shown below. This matrix includes all the outgroup taxa and the reconstructed ancestor. Morphological data are given at the end and were used as they were used in Reeder and Montanucci (2001).

#NEXUS Begin DATA; Dimensions ntax=20 nchar=2542; Format gap=- MISSING=? EQUATE="N=?" EQUATE="R={A,G}" EQUATE="Y={C,T}" EQUATE="M={A,C}" EQUATE="W={A,T}" EQUATE="S={C,G}" EQUATE="K={G,T}" EQUATE="D={A,G,T}" EQUATE="H={A,C,T}" EQUATE="V={A,C,G}" EQUATE="B={C,G,T}" EQUATE="Z={T,-}" EQUATE="X={G,-}" EQUATE="E={A,-}" EQUATE="F={C,-}" EQUATE="J={A,C,-}" EQUATE="P={T,C,-}" SYMBOLS="A~Z 0~9";

Matrix [12S rRNA = 1-252, 16S rRNA = 253-713, cytb = 714-1757, nd4 = 1758-2510, morphology = 2511-2542]

```
C.draconoides  CCGCCAGAAAATTACGAGCGAAAAGCTTAAAACTCAAAGGACT
C.texanus      CCGCCAGAAAATTACGAGCGAAAAGCTTAAAACTCAAAGGACT
H.maculata     CCGCCAGAAAATTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.asio         CCGCCAGAAGACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.braconnieri  ??????????????????????????????????????????
P.cornutum     CCGCCAGAAGACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.coronatum    ???CCAGAA?ATTACAAGC?AAAAGCTTAAAACTCAAAGGACT
P.ditmarsi     CCGCCAGAAAACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.douglasii    CCG?CAGAAAACTACAAGCGAAAAGCTTAAAACTCAAAGGACT
P.hernandesi   ??????????????????????????????????????????
P.mcallii      CCGCCAGAAAACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.modestum     TCGCCAGAAAACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.orbiculare   CCGCCAGAAAACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.platyrhinos  CCGCCAGAAAATTACGAGCGAAGAGCTTAAAACTCAAAGGACT
P.solare       CCGCCAGAAGACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
P.taurus       CCGCCAGAAAACTACGAGCGAAAAGCTTAAAACTCAAAGGACT
S.merriami     CCGCCAGAAAATTACGAGCAAAAAGCTTAAAACTCAAAGGACT
U.ornatus      CCGCCAGAAAATTACGAGCGAAAAGCTTAAAACTCAAAGGACT
U.stansburiana CCGCCAGAAGATTACGAGCGAAAAGCTTAAAACTCAAAGGACT
Ancestor       CCGCCAGAAAATTACGAGCGAAAAGCTTAAAACTCAAAGGACT

C.draconoides  TGGCGGTGCTCCATATCCGACTTAGAGGAGCCTGTCCTATAAT
C.texanus      TGGCGGTGCTCCACACCCGGCTTAGAGGAGCCTGTCCTATAAT
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H.maculata      TGGCGGTGC-CCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.asio          TGGCGGTGCTCCAT--CCGACTTAGAGGAGCCTGTCCTATAAT
P.braconnieri   ??????????????????????????????????????????
P.cornutum      TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.coronatum     TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.ditmarsi      TGACGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.douglasii     TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.hernandesi    ??????????????????????????????????????????
P.mcallii       TGGCGGTGCTCCACGCCCGACTTAGAGGAGCCTGTCCTATAAT
P.modestum      TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.orbiculare    TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.platyrhinos   TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.solare        TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT
P.taurus        TGGCGGTGCTTCACACCCGACTTAGAGGAGCCTGTCCTATAAT
S.merriami      TGGCGGTTCTCCACA-CCAACTTAGAGGAGCCTGTCCTATAAT
U.ornatus       TGGCGGTGCCCCAAA-CCAACTTAGAGGAGCCTGTCCTATAAT
U.stansburiana  TGGCGGTGCTCCATA-CCGACTTAGAGGAGCCTGTCCTATAAT
Ancestor        TGGCGGTGCTCCACACCCGACTTAGAGGAGCCTGTCCTATAAT

C.draconoides   CGATAATCCACGTTAAACCTCACCATTCATTGCC-CAGCCTAT
C.texanus       CGATACTCCACGATAAACCTCACCACTCATTGCC-CAGCCTAT
H.maculata      CGATACTCCACGCTAAACCTCACCATTCATTGCC-CAGCCTAT
P.asio          CGATACTCCACGCTAAACCTTACCATTCATTGCC-CAGCCTAT
P.braconnieri   ??????????????????????????????????????????
P.cornutum      CGATACTCCACGTTAGACCTTTCCACTCATTGCCTCAGCCTAT
P.coronatum     CGATACTCCACGCTAAACCTCACCATTCATTGCC-CAGCCTAT
P.ditmarsi      CGATACTCCACGCTAAACCCCACCACTCATTGCC-CAGCCTAT
P.douglasii     CGATACTCCACGCTAAACCCCACCACTCATTGCC-CAGCCTAT
P.hernandesi    ??????????????????????????????????????????
P.mcallii       CGATACTCCACGCTAAACCCCACCACTCATTGCC-CAGCCTAT
P.modestum      CGATACTCCACGCTAAACCTCACCACTCATTGCC-CAGCCTAT
P.orbiculare    CGATACTCCACGCTAAACCCAACCACTCATTGCC-CAGCCTAT
P.platyrhinos   CGATACTCCACGCTAAACCCCACCATTCATTGCC-CAGCCTAT
P.solare        CGATACTCCACGATAAACCTCACCACCCATTGCC-CAGCCTAT
P.taurus        CGATACTCCACGCTAAACCTCACCATTCATTGCC-CAGCCTAT
S.merriami      CGATACCCCACGCTAAACCTCACCAATCTTAGCCTCAGCCTAT
U.ornatus       CGATACTCCACGATAAACCTAACCAATTTTAGCC-CAGCCTAT
U.stansburiana  CGATACTCCACGCTAAACCTCACCACTCTTTGCC-CAGCCTAT
Ancestor        CGATACTCCACGCTAAACCTCACCAYTCATTGCCTCAGCCTAT

C.draconoides   ATACCGCCGTCGTCAACTTACCCCATGAGGGATCAACAGTAAG
C.texanus       ATACCGCCGTCGACAACTTACCCCATGAGGGCATAACAGTAGG
H.maculata      ATACCGCCGTCGCCAACTTACCCCCTGAGGGTAAAACAGTAAG
P.asio          ATACCGCCGTCGCCAACTTACCCCATGAGGACTCAACAGTAAG
P.braconnieri   ??????????????????????????????????????????
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P.cornutum      ATACCGCCGTCGCCAACCTACCCCATGAGGGTTCAATAGTAGG
P.coronatum     ATACCGCCGTCACCAACTTACCCCATGAGGGTTAAACAGTAAG
P.ditmarsi      ATACCGCCGTCGCCAACTTACCCCATGAGGGCTTAACAGTAAG
P.douglasii     ATACCGCCGTCACCAACTTACCCCATGAGGGCTTAACAGTAAG
P.hernandesi    ??????????????????????????????????????????
P.mcallii       ATACCGCCGTCACCAACTTACCCCATAAGGGTCCAACAGTAGG
P.modestum      ATACCGCCGTCGCCAACTTACCCCATGAGGGTTCAACAGTAAA
P.orbiculare    ATACCGCCGTCGCCAACTTACCCCATAAGGGTC-AACAGTAAG
P.platyrhinos   ATACCGCCGTCGCCAACTTACCCCATGAGGGCCAAACAGTAAG
P.solare        ATACCGCCGTCGCCAACTTACCCCATGAGGGCTTAATAGTAAG
P.taurus        ATACCGCCGTCACCAACTTACCCCATGAGGGCTCAACAGTAAA
S.merriami      ATACCGCCGTCACCAATCTACCTCGTGAGAGAAAAACAGTAAG
U.ornatus       ATACCGCCGTCGCCAACCTACTCCCTGAGGAAAAAACAGTAAG
U.stansburiana ATACCGCCGTCGCCAACTTACCCCATGAGGGAAAAACAGTAGG
Ancestor        ATACCGCCGTCGCCAACTTACCCCATGAGGGHAAAACAGTAAG

C.draconoides   TATAATAGTACTAAAACGTCAGGTCAAGGTGTAGCTTATAGAA
C.texanus       TAAAACAGTACTAAAACGT-AGGTCAAGGTGTAGCTTATAGAG
H.maculata      TACAATAGTACTAAAACGTCAGGTCAAGGTGTAGCTTATAGAA
P.asio          TACAACAGTACTAAAACGTCAGGTCAAGGTGTAGCTTATAGAA
P.braconnieri   ??????????????????????????????????????????
P.cornutum      TTCAATAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.coronatum     TACAACAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAA
P.ditmarsi      TACAATAGCACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.douglasii     TACAATAGCACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.hernandesi    ??????????????????????????????????????????
P.mcallii       TACAACAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.modestum      TATAATAGT-CTAGAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.orbiculare    TACAATAGCGCTAAAACGTCAGGTCAAGGTGTAGCTAATAGAG
P.platyrhinos   TATAACAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAA
P.solare        TACAATAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGGG
P.taurus        TAAAATAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAGAA
S.merriami      TACAAAAGTACTAAAACGTCAGGTCAAGGTGTAGCTAACAGAA
U.ornatus       TAAAAAAGTACTAAAACGTCAGGTCAAGGTGTAGCTAATAAAT
U.stansburiana TAAAAAAGT-CTAATACGTCAGGTCAAGGTGTAGCTAATAGAC
Ancestor        TAAAAYAGTACTAAAACGTCAGGTCAAGGTGTAGCTWATAGAR

C.draconoides   TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
C.texanus       TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
H.maculata      TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.asio          TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.braconnieri   ??????????????????????????????????????????
P.cornutum      TGGT-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.coronatum     TGGC-AGAGATGGGCTACATTTTTTCCGCGGTATTCTAACCGT
P.ditmarsi      TGGT-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
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```
P.douglasii     TGGC-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.hernandesi    ????????????????????????????????????????
P.mcallii       TGGT-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.modestum      TGGC-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.orbiculare    TGGT-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.platyrhinos   TGGC-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.solare        TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
P.taurus        TGGC-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
S.merriami      TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
U.ornatus       TGGA-AGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT
U.stansburiana  TGGTTAGAGATGGGCTACATTTTT-CCGCGGTATCCTAACCGT
Ancestor        TGGATAGAGATGGGCTACATTTTTTCCGCGGTATCCTAACCGT

C.draconoides   GCAAAGGTAGCGTAA?CACTTGT?TCCTAAATAGAGACCTGTA
C.texanus       GCAAAGGTAGCGTAATCATTTGTCCCCTAAATAGAGACCTGTA
H.maculata      GCAAAGGTAGCGTAA-CACTTGTCTCCTAAATAGAGACCTGTA
P.asio          GCAAAGGTAGCGTAATCACTTGTCCCCTAAATAGAGACCTGTA
P.braconnieri   ????????????????????????????????????????
P.cornutum      GCAAAGGTAGCGTAA?CACTTGTCCCCTAAATAGAGACTCGTA
P.coronatum     GCAAAGGTAGCGTAA?CACTTGTCCCCTAAATAGGGACCTGTA
P.ditmarsi      GCAAAGGTAGCGTAATCACTTGTCTCCTAAATAGGGACTAGTA
P.douglasii     GCAAAGGTAGCGTAATCACTTGTCCCCTAAATAGAGACTAGTA
P.hernandesi    ????????????????????????????????????????
P.mcallii       GCAAAGGTAGCGTAATCACTTGTCCCCTAAATAGAGACCTGTA
P.modestum      GCAAAGGTAGCGTAA?CACTTGTCCCCTAAATAGAGACCAGTA
P.orbiculare    GCAAAGGTAGCGTAATCACTTGTCTCCTAAATAGGGACCAGTA
P.platyrhinos   GCAAAGGTAGCGCAATCACTTGTCCCCTAAATAGAGACCTGTA
P.solare        GCAAAGGTAGCGTAATCACTTGTCTCCTAAATAGAGACCAGTA
P.taurus        GCAAAGGTAGCATAATCACTTGTCTTCTAAATAAAGACCTGTA
S.merriami      GCAAAGGTAGCGTAA-CACTTGCCCTTTAAATAAGGGCCCGTA
U.ornatus       GCAAAGGTAGCGTAA?CACTTGTCCTCCAAATAGGGACCTGTA
U.stansburiana  GCAAAGGTAGCGTAA?CACTTGTCCCCTAAATAGGGACCTGTA
Ancestor        GCAAAGGTAGCGTAATCACTTGTCCCCTAAATAGRGACCTGTA

C.draconoides   TGAATGGCTAAATGAGGTCCAACCTGTCTCCTTTAATTAATCA
C.texanus       TGAATGGCTAAATGAGGACTAACCTGTCTCCTTTAATTAATCA
H.maculata      TGAACGGCTAAATGAGGACTAA?CTGTCTCCTT?AA??AATCA
P.asio          TGAATGGCTAAATGAGGACTTAACTGTCTCCTTTAATTAATCA
P.braconnieri   ????????????????????????????????????????
P.cornutum      TGAATGGCTAAATGAGGATTAATCTGTCTCCTTTAATTAATCA
P.coronatum     TGAATGGCTAAATGAGGACCAGACTGTCTCCTTTAATTAATCA
P.ditmarsi      TGAACGGCTAAATGAGGACCAATCTGTCTCCTTTAATTAATCA
P.douglasii     TGAACGGCTAAATGAGGACTAATCTGTCTCCTTTAATTAATCA
P.hernandesi    ????????????????????????????????????????
P.mcallii       TGAATGGCTAAATGAGGACTAAACTGTCTCTTTTTATTAATCA
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P.modestum      TGAACGGCTAAATGAGGACTAA?CTGTCTCCTTTAATCAATCA
P.orbiculare    TGAACGGCTAAATGAGGACTAATCTGTCTCCTTTAATTAATCA
P.platyrhinos   TGAACGGCTAAATGAGGACTAAACTGTCTCTTTTTATTAATCA
P.solare        TGAACGGCTAAATGAGGACTTATCTGTCTCTTCTAATTAATCA
P.taurus        TGAACGGCTAAATGAGGGTTAATCTGTCTCCTTTAATTAATCA
S.merriami      TGAATGGCTAAATGAAGATTAATCTGTCTCCTTTAATAAATCA
U.ornatus       TGAACGGCTAAATGAGGACCAATCTGTCTCCTTTGATTAA?CA
U.stansburiana TGAACGGCTAAATGAGGACTAA?CTGTCTCCTCTAACCAGTCA
Ancestor        TGAAYGGCTAAATGAGGACTAATCTGTCTCCTTTAATYAATCA

C.draconoides   GTGAAACTGATCTACCAGTACAAAAGCTGGTATACTATCATTA
C.texanus       GTGAAACTGATCTGCCAGTACAAAAGCTGGTATATAATCATTA
H.maculata      GTGAAACTGATCTACCAGTACAAAAGCTGGTATTTTACCATTA
P.asio          GTGAAACTGATCTTCCAGTACAAAAGCTGGCATAATATCATAA
P.braconnieri   ??????????????????????????????????????????
P.cornutum      GTGAAACTGATCTACCAGTACAAAAGCTGGTATTTTATCATAA
P.coronatum     GTGAAACTGATCTACCAGTCCAAAAGCTGGTATTTCATCATAA
P.ditmarsi      GTGAAACTGATCTACCAGTACAAAAGCTGGTATTATATCATAA
P.douglasii     GTGAAACTGATCTACCAGTACAAAAGCTGGTATTAAACCATAA
P.hernandesi    ??????????????????????????????????????????
P.mcallii       GTAAAACTGATCTACCAGTACAAAAGCTGGTATTTTATCATAA
P.modestum      GTGAAACTGATCTACCAGTACAAAAGCTGGTATAATATCATAA
P.orbiculare    GTGAAACTGATCTACCAGTACAAAAGCTGGTATTATATCATAA
P.platyrhinos   GTAAAACTGATCTACCAGTACAAAAGCTGGTGTTTTATCATAA
P.solare        GTGAAACTGATCTACCAGTACAAAAGCTGGCATTTTACCATAA
P.taurus        GTGAAACTGATCTACCAGTACAAAAGCTGGTATTCTACCATAA
S.merriami      GTGAAACTGATCTACCAGTACAAAAGCTGGTATAAAACCATAA
U.ornatus       GTGAAACTGATCTACCAGTACAAAAGCTGGTATAACTCCATAA
U.stansburiana GTGAAACTGATCTGCCAGTACAAAAGCTGGCATTTCTTCATAA
Ancestor        GTGAAACTGATCTACCAGTACAAAAGCTGGTATATHAYCATAA

C.draconoides   GACGAGAAGACCCTGTGGAGCTTTAAATCTTGGCCCAAGATTT
C.texanus       GACGAGAAGACCCTGTGGAGCTTTAAATCTTGGTTCAAGATTT
H.maculata      GACGAGAAGACCCTGTGGAGCTTTAAATCTTGGCCCAAGATTT
P.asio          GACGAGAAGACCCTGTGGAGCTTTAAATCATGGCTTAAGACTT
P.braconnieri   ??????????????????????????????????????????
P.cornutum      GACGAGAAGACCCTGTGGAGCTTTAAATTGTGGCCTAAAATTT
P.coronatum     GACGAGAAGACCCTGTGGAGCTTTAAATTTTGGCCCAAAATTT
P.ditmarsi      GACGAGAAGACCCTGTGGAGCTTTAAATTATGGCCCAAGATTT
P.douglasii     GACGAGAAGACCCTGTGGAGCTTTAAATTATGGCCCAAAATTT
P.hernandesi    ??????????????????????????????????????????
P.mcallii       GACGAGAAGACCCTGTGGAGCTTTAAATTATGGCCTAAAATTT
P.modestum      GACGAGAAGACCCTGTGGAACTTTAAATTTTGGTCTAAAATTT
P.orbiculare    GACGAGAAGACCCTGTGGAGCTTTAAATTGTGGTCCAAAATTT
P.platyrhinos   GACGAGAAGACCCTGTGGAGCTTTAAATTATGGCCTAAAATTT
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P.solare        GACGAGAAGACCCTGTGGAGCTTTAAATTTTGGCTAAAAATTT
P.taurus        GACGAGAAGACCCTGTGGAGCTTAAAATTATGACCAAAAATTT
S.merriami      GACGAGAAGACCCTGTGGAGCTTTAAATTCTGGC?A-AAATTT
U.ornatus       GACGAGAAGACCCTGTGGAGCTTTAAACTTTGGCAAAAAGTTT
U.stansburiana GACGAGAAGACCCTGTGGAGCTTTAAATTTTGGCCCAAAATTT
Ancestor        GACGAGAAGACCCTGTGGAGCTTTAAATYTTGGCCCAARATTT

C.draconoides   TAAGTTGGGGCGACTTCGGAACAAAACAAAACTCCCGAGCATA
C.texanus       TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
H.maculata      TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCACA
P.asio          TAAGTTGGGGCGACTTCGGAACAAAATATAACTTCCGAGCATA
P.braconnieri   ??????????????????????????????????????????
P.cornutum      TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.coronatum     TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.ditmarsi      TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.douglasii     TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.hernandesi    ??????????????????????????????????????????
P.mcallii       TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.modestum      TAAGTTGGGGCAACTTCGGAACAAAACAAAACTTCCGAGCATA
P.orbiculare    TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.platyrhinos   TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.solare        TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
P.taurus        TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA
S.merriami      TAAGTTGGGGCGACTTCGGAAAAAAACAAAACTTCCGAGCATA
U.ornatus       TAAGTTGGGGCGACTTCGG?ATAAAAAAAAACTTCCGAGCATA
U.stansburiana TAAGTTGGGGCGACTTCGGAAAAAAACAAAACTTCCGAGCATA
Ancestor        TAAGTTGGGGCGACTTCGGAACAAAACAAAACTTCCGAGCATA

C.draconoides   A-GGATAACCTTACCAAGACCAACAAGTCAAAACAAAAATTGA
C.texanus       A-GGACTACCTAACCAAGACCCACAAGTCAAAACAAAA-CTGA
H.maculata      A-GGACAACCTTATTAAGACCAACAAGTCAAAACAAAAATTGA
P.asio          AAGGATAACCTCACCAAGACCTACAAGTCAAAGCAAAACTTGA
P.braconnieri   ??????????????????????????????????????????
P.cornutum      AAGGATAACCTTATCAAGACCTACAAGTCAAAACAAAACTTGA
P.coronatum     AAGGATAACCTCATCAAGACCCACAAGTCAAAACAAAACTTGA
P.ditmarsi      AAGGTACACCTCACCAAGACTAACAAGTCAAAGCAAAACCAGA
P.douglasii     AAGGTACACCTCACCAAGACTAACAGGTCAAAACAAAACTAGA
P.hernandesi    ??????????????????????????????????????????
P.mcallii       AAGGATTTCCTCACCTAGACCAACAAGTCAAAGCAAAAATCGA
P.modestum      AAGGATAACCTTACCAAGACAAACAAGTCAAAACAAAACTAGA
P.orbiculare    AAGGAA-ACCTCACCAAGACTAACAAGTCAAAACAAAACTAGA
P.platyrhinos   AAGGATCCCCTCACCTAGACCAACAAGTCAAAGCAAAACTTGA
P.solare        AAGGATCACCTTTCCAAGACTAACACGTCAAAGCAAAAATAGA
P.taurus        AAGGATTACCTTAACAAGACCAACAAGTCTAAACAAAACTAGA
S.merriami      A-GATCA-TCTAACCAAGACCAACAAGTCAAAGCAAAACTTGA
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U.ornatus       AAGAAAAATCTTACCAAGACCTACAAGTCAAAGCAAAACTTGA
U.stansburiana AAGAATAATCTTACCAAGACCAACAAGTCAAAGCAAAATTAGA
Ancestor        AAGGAYAACCTTACCAAGACCAACAAGTCAAARCAAAAMTTGA

C.draconoides   CCCAGTACAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
C.texanus       CCCAGTACAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
H.maculata      CCCAGTATTA--CTGATCACCGAACCAAGTTACCCCAGGGATA
P.asio          CCCAGTATAA--CTGATCATCGAACCAAGTTACCCCAGGGATA
P.braconnieri   ??????????????????????????????????????????
P.cornutum      CCCAGTACAA--CTGAGCACCGAACCAAGTTACCCCAGGGATA
P.coronatum     CCCAGTATAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
P.ditmarsi      CCCAGTACAA--CTGACCACCGAACCAAGTTACCCCAGGGATA
P.douglasii     CCCAGTACAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
P.hernandesi    ??????????????????????????????????????????
P.mcallii       CCCAGTACACA-CTGATCACCGAACCAAGTTACCCCAGGGATA
P.modestum      CCCAGTAAAA--CTGACCACCGAACCAAGTTACCCCAGGGATA
P.orbiculare    CCCAGTATAA--CTGATTACCGAACCAAGTTACCCCAGGGATA
P.platyrhinos   CCCAGTATAAAACTGACCACCGAACCAAGTTACCCCAGGGATA
P.solare        CCCAGTAAAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
P.taurus        CCCAGTACAA--CTGATCACCGAACCAAGTTACCCCAGGGATA
S.merriami      CCCAGTAAAA--CTGACTACCGAACCAAGTTACCCCAGGGATA
U.ornatus       CCCAGTAA?A--CTGATCAC?GAACCAAGTTACCCCAGGGATA
U.stansburiana CCC??TAAAA--CTGATCATCGAACCAAGTTACCCCAGGGATA
Ancestor        CCCAGTAMAAAACTGATCACCGAACCAAGTTACCCCAGGGATA

C.draconoides   ACAGCGCAATCTTCTTCAAGAGTTCTTATCGACAAGAAGGTTT
C.texanus       ACAGCGCAATCTTCTTCAAGAGTTCTTATCGACAAGAAGGTTT
H.maculata      ACAGCGCAATCTTCTTCAAGAGTTCCCATCGACAAGAAGGTTT
P.asio          ACAG?GCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
P.braconnieri   ??????????????????????????????????????????
P.cornutum      ACAGCGCAATCTTCTTCAAGAGTTCATATCGACAAGAAGGTTT
P.coronatum     ACAGCGCAATCTTCTTCAAGAGTTCATATCGACAAGAAGGTTT
P.ditmarsi      ACAGCGCAATCTTCTTCAAGAGTTCACATCGACAAGAAGGTTT
P.douglasii     ACAGCGCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
P.hernandesi    ??????????????????????????????????????????
P.mcallii       ACAGCGCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
P.modestum      ACAGCGCAATCTTCTTCAAGAGTTCATATCGACAAGAAGGTTT
P.orbiculare    ACAGCGCAATCTTCTTCAAGAGTTCATATCGACAAGAAGGTTT
P.platyrhinos   ACAGCGCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
P.solare        ACAGCGCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
P.taurus        ACAGCGCAATCTTCTTCAAGAGTTCATATCGACAAGAAGGTTT
S.merriami      ACAGCGCAATCTTCTTCAAGAGTCCATATCGACAAGAAGGTTT
U.ornatus       ACAGCGCAATCTTCTTCAAGAGTCCCTATCGACAAGAAGGTT?
U.stansburiana ACAG?GCAATCCTCTTCAAGAGTCCATATCGACAAGAGGGTT-
Ancestor        ACAGCGCAATCTTCTTCAAGAGTYCATATCGACAAGAAGGTTT
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C.draconoides  ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCCGC
C.texanus      ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCCGC
H.maculata     ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCCGC
P.asio         ACGACCTCGATGTTGGATCAGGACACCCAAACGGTGCAGCAGC
P.braconnieri  ??????????????????????????????????????????
P.cornutum     ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGAAGC
P.coronatum    ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCAGC
P.ditmarsi     ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCCGC
P.douglasii    ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAACAGC
P.hernandesi   ??????????????????????????????????????????
P.mcallii      ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAACAGC
P.modestum     ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAACAGC
P.orbiculare   ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCAGC
P.platyrhinos  ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAACAGC
P.solare       ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCAGC
P.taurus       ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCAGC
S.merriami     ACGACCTCGATGTTGGATCAG-ACACCCAAATGGTGCAACC--
U.ornatus      ????CCTCGATGTTGGATCAG?ACACCCAAATGGTGCAGCCGC
U.stansburiana ----------------------------------------
Ancestor       ACGACCTCGATGTTGGATCAGGACACCCAAATGGTGCAGCCGC

C.draconoides  TATTAAA--TTTGGATCACTACTGGGAATTTGTCTAATTATCC
C.texanus      TATTAAAGGTTTGGCTCACTGCTAGGAATTTGCCTAATTATCC
H.maculata     TATTAAAGGTTCGGCTCATTATTAGGAATTTGCTTAATTATCC
P.asio         TGTTAAAGGTTCGGATCACTACTAGGAGTATGCCTAATCATTC
P.braconnieri  ?????????TTTGGCTCACTACTAGGAATTTGCCTAATTATTC
P.cornutum     TATTAAAGGTTCGGCTCACTCCTAGGAATCTGCCTAATTATCC
P.coronatum    TATTAAAGGTTCGGCTCACTACTTGGAATTTGCCTAATTATTC
P.ditmarsi     TATTAAAGGTTTGGCTCTCTACTAGGAATCTGCTTAATTATCC
P.douglasii    TATTAAAGGTTTGGCTCACTACTGGGAATTTGCTTAATTACTC
P.hernandesi   ?????????TTTGGTTCGCTACTGGGAATCTGCTTAATTATCC
P.mcallii      TATTAAA?GTTCGGCTCATTACTCGGAATTTGTTTAATTGTTC
P.modestum     TATTAAAGGTTTGGTTCACTACTAGGAATCTGCTTAATTATTC
P.orbiculare   TATTAAAGGTTTGGCTCACTGCTTGGAATCTGCTTAATTATTC
P.platyrhinos  TATTAAAGGTTCGGCTCATTATTAGGAATCTGTTTAATTATTC
P.solare       TATTAAAGGTTTGGCTCACTACTAGGAACATGTTTAATTATTC
P.taurus       TATTAAAGGTTTGGTTCACTATTAGGAATTTGCCTAATTATTC
S.merriami     ---------TTTGGTTCACTCCTAGGAGCATGCCTAATTATCC
U.ornatus      TATTAAA--TTTGGATCCCTCCTAGGCATATGCCTAATTATTC
U.stansburiana ---------TTTGGTTCCCTTCTAGGACTTTGCTTAATTATCC
Ancestor       TATTAAAGGTTTGGHTCACTMCTAGGAATTTGCCTAATTATCC

C.draconoides  AAATCCTAACAGGACTATTTCTAGCCATACACTATACAGCTGA
C.texanus      AAATCCTAACAGGACTATTTTTAGCAATACACTATACAGCCGA
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H.maculata      AAATTTTAACAGGTCTATTCTTAGCCATACACTACACAGCTGA
P.asio          AAACCCTAACAGGCCTATTTCTGGCCATACACTACACAGCCGA
P.braconnieri   AAATCTTAACAGGCCTTTTCCTCGCCATACATTACACAGCCGA
P.cornutum      AAATCTTAACAGGCTTATTTTTAGCTATACACTACACAGCTGA
P.coronatum     AAATTCTCACAGGCCTATTTCTCGCCATACATTATACAGCTGA
P.ditmarsi      AAATCCTAACAGGCCTTTTTCTCACCATACACTACACGGCCGA
P.douglasii     AAGTCTTAACAGGTCTTTTCCTCGCCATGCACTACACAGCCGA
P.hernandesi    AAATCCTAACAGGCCTTTTTCTCGCCATACATTACACAGCCGA
P.mcallii       AAGTCCTTACAGGCCTTTTCCTCGCCATACACTACACAGCTGA
P.modestum      AAATCCTCACAGGCCTTTTTCTCGCCATGCACTACACAGCCGA
P.orbiculare    AAGTTCTGACAGGCCTTTTTCTAGCCATACACTATACAGCCGA
P.platyrhinos   AAATCATTACAGGCCTTTTTCTCGCTATACACTACACAGCCGA
P.solare        AAATCCTCACAGGACTATTTCTTGCCATACACTACACAGCTGA
P.taurus        AAATTCTAACAGGCCTTTTCCTCGCCATACACTACACAGCCGA
S.merriami      AAATCTTAACTGGTCTCTTCTTAGCTATACACTACACAGCTGA
U.ornatus       AAATCATAACCGGACTTTTCCTAGCCATACACTACACAGGAGA
U.stansburiana  AAATCCTAACAGGATTATTCCTAGCAATACACTATACAGCCGA
Ancestor        AAATCCTAACAGGACTATTYCTAGCCATACACTATACAGCTGA

C.draconoides   CATTACATCAGCCTTTTCATCCGTTGCTCACATCTGCCGAGAT
C.texanus       CATTACATCAGCCTTCTCATCAATCGCCCACATTTGCCGAGAT
H.maculata      CATTACATCAGCCTTTTCATCAGTAGCCCACATTTGTCGAGAC
P.asio          CATTTCATCTGCCTTCTCATCCGTAGCTCATATCTGCCGAGAC
P.braconnieri   CATTACATCCGCCTTTTCATCCGTAGCCCATATCTGCCGAGAT
P.cornutum      CATTACATCCGCTTTTTCATCCGTAGCTCACATCTGTCGAGAC
P.coronatum     CATTATATCCGCCTTCTCATCCGTAGCCCACATCTGCCGAGAC
P.ditmarsi      CACTATGTCCGCCTTTTCATCTGTGGCTCATATCTGCCGAGAT
P.douglasii     CATTACATCCGCCTTTTCATCTGTAGCCCATATCTGCCGAGAC
P.hernandesi    CATTATGTCCGCTTTTTCATCTGTAGCTCACATCTGCCGAGAT
P.mcallii       CATTTCATCTGCCTTTTCATCCGTAGCCCACATTTGCCGAGAT
P.modestum      CATTTCATCCGCCTTCTCATCTGTAGCTCACATCTGCCGAGAC
P.orbiculare    CATTACATCAGCCTTCTCATCCGTAGCCCACATCTGCCGAGAT
P.platyrhinos   CATTTCGTCCGCCTTCTCATCCGTAGCCCACATTTGCCGAGAT
P.solare        TATTTCATCCGCCTTCTCATCTGTAGCCCACATCTGTCGAGAT
P.taurus        CATCACATCCGCCTTTTCATCCGTATCTCACATCTGCCGAGAC
S.merriami      TATTTCCTCAGCTTTCTCATCCATTACCCACATCTGCCGAGAT
U.ornatus       TATCTCATCTGCCTTCTCATCAGTAGCCCACATTTGTCGAGAT
U.stansburiana  TATTTCATCAGCATTTTCATCAATTGCTCACATTTGTCGAGAT
Ancestor        YATTWCATCAGCCTTTTCATCMGTWGCYCACATTTGYCGAGAT

C.draconoides   GTACAATATGGCTGACTTATCCGAAATATTCATGCCAACGGAG
C.texanus       GTCCAATATGGCTGACTTATCCGAAACATCCACGCTAACGGAG
H.maculata      GTACAATACGGCTGACTTATCCGAAATATCCATGCCAACGGCG
P.asio          GTACAGTACGGATGACTCATCCGAAACATCCATGCAAACGGCG
P.braconnieri   GTCCAATACGGCTGACTTATCCGAAATATTCATGCCAACGGTG
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P.cornutum      GTACAATACGGCTGACTAATCCGAAACATTCATGCCAACGGCG
P.coronatum     GTTCAATATGGATGACTCATCCGAAATATTCACGCCAACGGCG
P.ditmarsi      GTACAATACGGCTGACTCATCCGCAATATTCACGCTAACGGCG
P.douglasii     GTTCAATACGGCTGACTTATCCGCAATATCCATGCCAACGGCG
P.hernandesi    GTTCAATATGGCTGACTTATCCGCAATATTCACGCTAACGGCG
P.mcallii       GTTCAACATGGCTGACTAATCCGAAACATTCACGCCAATGGCG
P.modestum      GTCCAATATGGCTGACTTATCCGCAACATTCACGCCAATGGCG
P.orbiculare    GTTCAATATGGGTGATTAATCCGAAACATTCACGCCAACGGCG
P.platyrhinos   GTTCAGTATGGCTGACTAATCCGAAATATTCATGCCAACGGCG
P.solare        GTACAATACGGGTGACTCATCCGAAACATCCACGCCAACGGAG
P.taurus        GTCCAACATGGCTGACTCATTCGAAATATTCACGCCAACGGTG
S.merriami      GTACAATACGGCTGACTCATCCGAAATATACATGCCAACGGAG
U.ornatus       GTACAATACGGATGACTGATCCGAAACCTCCATGCAAACGGAG
U.stansburiana GTACAATACGGCTGACTCATCCGAAACACACATGCAAACGGAG
Ancestor        GTACAATAYGGCTGACTYATCCGAAAYATYCATGCMAACGGAG

C.draconoides   CCTCCATATTTTTTATCTGTATTTATCTCCACATTGGACGAGG
C.texanus       CCTCTATATTTTTCATTTGCATCTACCTTCACATTGGCCGAGG
H.maculata      CTTCCATATTCTTTATCTGCATTTATCTTCACATTGGTCGAGG
P.asio          CCTCCATATTCTTTATCTGTATTTACCTTCACATCGGCCGAGG
P.braconnieri   CTTCTATATTCTTCATCTGCATTTATCTTCACATCGGCCGAGG
P.cornutum      CCTCCATGTTCTTCATTTGTATTTACCTCCACATTGGCCGAGG
P.coronatum     CCTCCATATTTTTTATCTGCATTTACCTCCACATCGGCCGAGG
P.ditmarsi      CCTCCATATTCTTTATCTGCATTTATCTACACATTGGCCGAGG
P.douglasii     CCTCCATATTCTTTATCTGCATCTACCTACACATCGGCCGAAG
P.hernandesi    CCTCTATATTCTTTATCTGCATCTACTTACACATCGGCCGAGG
P.mcallii       CATCTATATTCTTCATCTGTATTTATCTCCACATTGGCCGAGG
P.modestum      CTTCCATATTTTTTATTTGCATTTATCTCCACATTGGTCGAGG
P.orbiculare    CCTCTATATTTTTTATCTGCATCTATTTTCACATCGGCCGAGG
P.platyrhinos   CATCCATATTTTTCATCTGCATTTATTTTCACATTGGCCGAGG
P.solare        CTTCCATATTTTTTATCTGCATCTATCTTCACATTGGCCGAGG
P.taurus        CCTCCATATTCTTCATCTGCATTTACCTACACATCGGCCGAGG
S.merriami      CTTCACTATTTTTTATCTGCATTTATTTTCACGTTGGCCGAGG
U.ornatus       CCTCAATATTCTTTATCTGCATCTATATGCATGTCGGACGAGG
U.stansburiana CTTCAATATTTTTTATCTGCATTTACATACATGTAGGACGAGG
Ancestor        CYTCCATATTTTTTATCTGCATTTATCTTCACATTGGHCGAGG

C.draconoides   AATATACTACGGATCTTATATGTTTAAAGAAACATGAAACATT
C.texanus       CCTATACTACGGATCATACATATTTAAAGAAACCTGAAATATT
H.maculata      CCTATACTATGGATCATACATATTTAAAGAAACATGAAATATT
P.asio          CCTCTACTACGGATCCTATATATTTAAAGAGACATGAAACATC
P.braconnieri   CCTTTATTATGGTTCCTATATATTTAAAGAAACATGAAACATT
P.cornutum      ATTATACTATGGCTCCTATATATTCAAAGAAACATGAAATATT
P.coronatum     TCTCTACTATGGATCTTACATGTTTAAAGAAACATGAAACATC
P.ditmarsi      CCTATATTATGGATCCTACATATTTAAAGAAACATGAAACATT
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P.douglasii     CCTATACTACGGATCCTATATATTCAAAGAAACATGGAACATC
P.hernandesi    CCTATATTATGGATCCTACATATTTAAAGAAACATGAAATATT
P.mcallii       CCTCTACTATGGATCCTATATATTTAAAGAAACATGAAACATC
P.modestum      CCTCTATTACGGATCCTACATATTTAAAGAGACATGAAATATC
P.orbiculare    CCTTTATTATGGATCCTATATATTTAAAGAAACATGAAACATT
P.platyrhinos   CCTCTACTATGGATCCTATATATTTAAAGAAACATGAAACATC
P.solare        CCTCTACTACGGCTCCTATATATTCAAAGAAACATGAAACATC
P.taurus        TATCTACTATGGCTCCTATATGTTTAAAGAAACATGAAACATT
S.merriami      CCTTTACTACGGATCCTACATATTTAAAGAGACCTGAAACATT
U.ornatus       CCTATACTACGGCTCATATATATTTAAAGAAACCTGAAACTTA
U.stansburiana  ACTTTACTACGGGTCATACATATTTAAAGAAACATGAAACCTT
Ancestor        MCTWTACTACGGATCWTACATATTTAAAGAAACATGAAACATT

C.draconoides   GGAGTAATCCTACTACTATTAGTCATAGCAACGGCATTCGTAG
C.texanus       GGAGTACTACTACTACTCCTAGTGATAGCGACAGCATTCGTAG
H.maculata      GGAGTAGTACTTTTACTGCTAGTTATAGCAACAGCATTCGTAG
P.asio          GGAGTAATCTTACTACTACTAGTCATAGCCACAGCATTCGTCG
P.braconnieri   GGAGTAACACTATTACTACTAGTTATAGCCACTGCATTCGTCG
P.cornutum      GGAGTAATTCTGCTACTACTATTAATAGCCACAGCATTCGTCG
P.coronatum     GGAGTAATCTTACTACTATTAGTTATAGCTACAGCATTTGTTG
P.ditmarsi      GGAGTAATTCTACTACTATTAGTCATAGCCACAGCATTCGTCG
P.douglasii     GGAGTAGTCCTATTACTTTTAGTTATAGCCACAGCATTCGTTG
P.hernandesi    GGAGTAATTCTGCTACTATTAATTATAGCCACAGCATTCGTCG
P.mcallii       GGAGTAGTTTTATTATTATTAGTTATAGCCACAGCATTCGTTG
P.modestum      GGAGTTATTCTATTACTACTAGTAATAGCCACAGCCTTCGTTG
P.orbiculare    GGTGTAGTACTATTATTATTAGTAATGGCTACAGCATTTGTCG
P.platyrhinos   GGAGTAATTTTACTATTATTAGTTATAGCCACAGCATTCGTTG
P.solare        GGAGTTATTTTATTACTATTAGTTATAGCTACAGCATTCGTTG
P.taurus        GGGGTAATATTATTATTACTAGTAATAGCCACCGCATTCGTTG
S.merriami      GGAGTAGCACTACTACTCCTAGTGATAGCTACAGCCTTTGTTG
U.ornatus       GGTGTTATTTTACTTCTGCTAGAAATAGCTACCGCCTTTGTCG
U.stansburiana  GGTGTTATTCTTCTACTTCTAGTAATAGCCACCGCCTTCGTAG
Ancestor        GGWGTWATHCTACTACT?CTAGTHATAGCYACMGCMTTCGTHG

C.draconoides   GGTACGTCTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
C.texanus       GCTACGTACTACCATGAGGACAAATATCATTCTGAGGGGCTGC
H.maculata      GTTATGTTTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.asio          GATATGTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.braconnieri   GGTACGTTTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.cornutum      GATACGTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.coronatum     GATACGTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.ditmarsi      GATACGTGTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.douglasii     GATACGTACTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.hernandesi    GATACGTACTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.mcallii       GTTATGTTTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
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P.modestum      GATATGTTTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.orbiculare    GATATGTATTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.platyrhinos   GCTATGTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.solare        GATATGTCTTACCATGAGGACAAATATCATTCTGAGGGGCTGC
P.taurus        GATATGTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
S.merriami      GCTATGTACTCCCATGAGGACAAATATCATTCTGAGGGGCTGC
U.ornatus       GGTACCTCCTACCATGAGGACAAATATCATTCTGAGGGGCTGC
U.stansburiana GATACGTACTGCCATGAGGACAAATATCATTCTGGGGGGCTGC
Ancestor        GSTACGTAYTACCATGAGGACAAATATCATTCTGAGGGGCTGC

C.draconoides   AGTTATAACTAATTTACTATCAGCTATTCCTTACGTAGGAACA
C.texanus       AGTTATTACCAACCTACTTTCAGCTATCCCATATATCGGTACA
H.maculata      AGTTATTACCAACCTACTCTCAGCCATCCCATATGTAGGAACA
P.asio          AGTTTATACAAACCTCCTATCGGCCATTCCATATATCGGATCA
P.braconnieri   AGTTATTACCAATCTCCTATCAGCCATTCCATATATTGGAGAG
P.cornutum      AGTATACACAAATCTATTATCAGCCATTCCATATGTTGGAACA
P.coronatum     AGTTTATACCAACCTTTTATCAGCCATTCCCTACATTGGAACA
P.ditmarsi      AGTTAACACTAATCTCTTATCTGCCATTCCCCATATCGGAACA
P.douglasii     AGTTATTACCAATCTTTTATCTGCTATTCCCTACATCGGAACA
P.hernandesi    AGTTAATACTAATCTTTTATCTGCCATTCCCTACATCGGAACA
P.mcallii       AGTTTATACCAACCTTTTATCACCTATTCCCTATATTGGAACA
P.modestum      AGTTTACACCAATCTTTTATCAGCTGTTCCTTACATCGGAACA
P.orbiculare    AGTTATTACTAATCTATTATCAGCCATCCCCTACGTTGGAACA
P.platyrhinos   AGTTATTACTAACCTTCTATCGGCAATTCCCTATATTGGAACA
P.solare        AGTAATTACTAACCTCCTATCAGCCATTCCCTACATCGGAACA
P.taurus        AGTTAATACCAACCTGCTATCGGCCATTCCATACATTGGAGGG
S.merriami      AGTTATCACCAATCTACTGTCAGCCATTCCATACATCGGCACC
U.ornatus       AGTAATTACTAATCTTCTATCAGCAATCCCATACATCGGAACA
U.stansburiana AGTTATTACCAACCTACTTTCAGCCGTCCCCTATATTGGAACA
Ancestor        AGTTATTACCAAYCTACTATCAGCYATCCCATAYRTYGGAACA

C.draconoides   ACCCTAGTAGAATGAATCTGAGGAGGATTCTCCGTCGACAACG
C.texanus       ACCCTAGTAGAGTGAATCTGAGGTGGCTTCTCCGTGGACAATG
H.maculata      ACCCTAGTAGAGTGAATCTGAGGGGGGTTTTCTGTCGACAACG
P.asio          ACCATAGTAGAATGAATCTGAGGGGGGTTTTCAATCGACAATG
P.braconnieri   ACCCTAGTAGAATGAGTCTGGGGGGGGTTCGCCGTTGACAACG
P.cornutum      ACCCTAGTAGAATGAATCTGAGGGGGATTCTCCGTAGATAATG
P.coronatum     ACCTTAGTAGAATGAATCTGAGGTGGCTTTTCCGTTGACAACG
P.ditmarsi      ACCCTAGTAGAATGAGTCTGAGGGGGGTTTTCTGTTGATAACG
P.douglasii     ACCTTAGTAGAATGAATCTGAGGTGGATTTTCTGTCGACAATG
P.hernandesi    GCCCTAGTAGAATGAATCTGAGGGGGTTTTTCTGTTGATAACG
P.mcallii       ACCATATTAGAATGAATCTGAGGGGGGTTTTCTGTTGATAACG
P.modestum      ACCCTAGTAGAATGAATCTGAGGTGGTTTCTCTGTAGACAACG
P.orbiculare    ACCCTAGTAGAATGAATCTGAGGGGGGTTTTCCATCGACAACG
P.platyrhinos   ACCATAGTAGAATGAATCTGAGGGGGGTTTTCTGTTGACAACG
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P.solare        ACCCTAGTAGAATGAATCTGAGGTGGGTTTTCCGTTGACAACG
P.taurus        ACCCTAGTAGAATGAGTCTGGGGGGGGTTCTCCGTTGATAACG
S.merriami      ACCATAGTTGAGTGAATCTGAGGGGGCTTTTCAGTAGATAACG
U.ornatus       ACCCTAGTAGAGTGAATCTGAGGAGGCTTCTCAGTTGACAACG
U.stansburiana ACCCTAGTTGAATGAATCTGAGGTGGATTTTCAGTTGACAACG
Ancestor        ACCCTAGTAGAATGAATCTGAGGKGGMTTYTCMGTYGACAACG

C.draconoides  CAACACTAACCCGATTCTTTACCTTTCACTTTCTCCTTCCATT
C.texanus       CAACACTAACCCGATTCTTTACATTTCACTTTCTACTTCCATT
H.maculata      CAACGTTAACCCGATTCTTTACATTTCACTTCCTCCTCCCATT
P.asio          CAACCCTTACCCGATTCTTTACCTTCCATTTTCTACTTCCATT
P.braconnieri   CAACCCTTACTCGATTTTTTACCTTTCATTTCCTACTACCATT
P.cornutum      CAACCCTCACCCGATTCTTCTCATTCCATTTTCTCCTACCATT
P.coronatum     CAACCCTAACCCGATTTTTTTCATTCCACTTCCTTTTACCATT
P.ditmarsi      CAACCCTGACCCGATTCTTTACATTCCACTTTCTCCTACCCTT
P.douglasii     CAACCCTAACCCGATTCTTTACATTCCACTTTCTTCTACCCTT
P.hernandesi    CAACCCTGACTCGATTCTTTACCTTCCATTTTCTCCTACCCTT
P.mcallii       CAACCCTCACTCGATTTTTTACATTCCATTTTCTCCTACCATT
P.modestum      CAACCCTCACTCGATTCTTCACATTTCACTTCCTACTACCATT
P.orbiculare    CAACCCTTACTCGATTTTTTACATTTCACTTCCTTTTACCTTT
P.platyrhinos   CGACACTCACTCGATTTTTTACATTTCACTTTCTTCTACCATT
P.solare        CAACCTTAACTCGATTTTTTACATTTCACTTCCTCCTACCCTT
P.taurus        CCACCCTTACTCGATTCTTCACCTTTCACTTCCTGTTACCATT
S.merriami      CAACCCTCACTCGATTCTTTACCTTCCACTTCCTACTACCATT
U.ornatus       CAACACTAACTCGATTTTTCACCTTTCATTTCCTTCTACCATT
U.stansburiana CAACACTAACCCGATTTTTTACCTTTCACTTCCTTCTTCCATT
Ancestor        CAACACTAACCCGATTYTTTACCTTTCACTTYCTYCTTCCATT

C.draconoides  CATCATTATTGGCATCACCATAATACATCTCCTATTTTTACAT
C.texanus       TGTTATCATTGGCGTTACCATAATACACCTTTTATTCCTACAC
H.maculata      TGCTATTATTGGTGTTACCCTAATACATCTCCTATTCCTACAC
P.asio          CACTATCATTGGAGCCTCAATAATACACCTTCTATTCCTACAT
P.braconnieri   CGCCATTATTGGAACCTCAATAATTCATCTACTATTTTTACAC
P.cornutum      TGCCATCATTGGTGCTTCAATAATTCACTTACTTTTTCTTCAT
P.coronatum     TGCCATTATTGGTGCTTCAATAATACACCTACTGTTTCTTCAC
P.ditmarsi      TACCATTATTGGTGCTTCAATAATACACCTATTGTTCTTACAT
P.douglasii     TGCCATTATTGCTACTTCAATAATACACCTATTATTTTTACAC
P.hernandesi    TGCTATTATTGGAGTTTCAATAATACACCTATTATTCTTACAT
P.mcallii       TGCCATCATTGGCGTTTCCATAATACACCTATTATTTTTACAT
P.modestum      TGCCATTATTGGCACCTCAATACTTCACCTTCTATTCCTACAC
P.orbiculare    CGCCATCATCGGTGCTTCAATAATACACCTATTATTCTTACAC
P.platyrhinos   TGCCATCATTGGGGTCTCTATAATGCACCTACTATTTTTACAT
P.solare        CGCTATCATTGGCGCCTCTATAATACACCTCCTATTCCTCCAT
P.taurus        CGCCATTATTGGAGCTTCAATAATACACCTTCTATTTCTACAC
S.merriami      TATCATTATTGGAGTATCCATAATACACCTTCTATTTTTACAT
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U.ornatus       CGCCATTATTGGAGTCTCCATAATTCACCTTCTTTTTCTCCAC
U.stansburiana TATTATCATCGGCGTTTCAATAATCCACCTCCTCTTTCTTCAT
Ancestor        TATYATTATTGGCGTYTCCATAATACAYCTCCTATTTCTACAT

C.draconoides   GAAACAGGCTCAAACAACCCAACTGGACTATCCTCAAACACAG
C.texanus       GAAACAGGATCTAACAACCCAACTGGATTAACCTCAAACACAG
H.maculata      GAAACAGGGTCTAATAACCCAACTGGACTAACCTCAAACTCAG
P.asio          GAAACCGGGTCAAACAACCCAACAGGACTAGCCTCAAACACAG
P.braconnieri   GAAACCGGGTCCAACAATCCAACAGGACTTACCTCAAACACAG
P.cornutum      GAAACCGGATCAAACAACCCAACAGGACTTTCCTCAAACACAG
P.coronatum     GAAACCGGATCAAACAACCCAACTGGACTCTCCTCAAATATAG
P.ditmarsi      GAAACAGGATCAAATAACCCAACCGGCCTTCCATCAAACACAG
P.douglasii     GAAACAGGATCAAATAACCCCACCGGTCTCTCCTCAAACACAG
P.hernandesi    GAAACAGGATCAAACAACCCAACCGGCCTCCCCTCAAACACAG
P.mcallii       GAAACAGGGTCAAACAACCCAACAGGACTCTCCTCAAATACAA
P.modestum      GAAACTGGATCAAACAACCCAACCGGCCTTTCCTCAAACACAG
P.orbiculare    GAAACCGGATCAAACAACCCAACCGGCCTCCCCTCAAACACAG
P.platyrhinos   GAAACAGGGTCAAACAACCCAACAGGACTCTCCTCAAACACAG
P.solare        GAAACTGGATCAAACAATCCAACAGGACTTCCCTCAAACACAG
P.taurus        GAAACCGGATCTAATAACCCAACAGGACTCACCTCAAACACAG
S.merriami      GAAACAGGCTCAAATAACCCAACAGGACTTAAATCAAACACAG
U.ornatus       GAAACCGGATCAAACAATCCAACAGGATTAACATCAAATACAG
U.stansburiana GAAACAGGCTCAAACAACCCAACAGGACTGGCCTCAAACACAG
Ancestor        GAAACAGGVTCAAACAACCCAACWGGACTAACCTCAAACACAG

C.draconoides   ACAAAGTTCCATTTCACCCATATTTTTCATACAAAGACCTCCT
C.texanus       ACAAAATTCCATTCCACCCATACTTTTCATATAAAGACTTACT
H.maculata      ATAAAGTCCCATTTCACCCATACTTTTCATACAAAGATCTTCT
P.asio          ACAAAGTCCCATTCCACCCATACTTTTCATACAAAGACATCCT
P.braconnieri   ACAAAATTCCTTTCCACCCATATTTTTCATACAAAGACCTCCT
P.cornutum      ACAAAGTCCCCTTCCACCCATACTTTTCATACAAAGACCTTCT
P.coronatum     ACAAAGTCCCATTCCACCCCTACTTTTCATATAAAGATCTCCT
P.ditmarsi      ACAAAGTTCCATTCCACCCCTACTTCTCATACAATGACTTAGT
P.douglasii     ATAAAGTTCCATTCCACCCATACTTCTCATATAAAGACTTATT
P.hernandesi    ACAAAGTTCCATTCCACCCATACTTCTCATATAAAGACCTACT
P.mcallii       ACAAAGTTCCATTCCACCCTTACTTCTCATATAAAAACCTTCT
P.modestum      ATAAAGTTCCATTCCACCCCTATTTTTCTTACAAGGATCTCCT
P.orbiculare    ATAAAATTCCATTCCATCCATACTTCTCATATAAAGACCTCCT
P.platyrhinos   ACAAAATTCCATTCCACCCCTACTTTTCATATAAAGATCTTCT
P.solare        ACAAAGTCCCATTTCACCCATACTTTTCATACAAAGACCTAAT
P.taurus        ACAAAATTTCATTCCACCCATATTTTTCATACAAAGACCTCCT
S.merriami      ACAAAGTACCGTTTCATCCATACTTCTCGTACAAAGACCTTCT
U.ornatus       ACAAAATCCCATTCCACCCGTATTTCTCATACAAAGACATACT
U.stansburiana ACAAAATCCCATTCCATCCATACTTTTCATATAAAGACCTATT
Ancestor        ACAAARTYCCATTYCAYCCATACTTTTCATACAAAGACCTACT
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C.draconoides  TGGAGCTTTACTACTAATTATTGTTCTACTAACCCTTGCACTA
C.texanus      CGGCGCCTTACTATTAATTATTATTCTACTAACACTCGCATTA
H.maculata     AGGCGCAATACTTTTAGTTATTCTTCTACTTCTACTTGCATTA
P.asio         AGGAATCCTTCTACTAACCATCACCTTATTATTATTAGCACTA
P.braconnieri  TGGCATCCTACTACTAATTATTATTTTATTATTACTTGCACTA
P.cornutum     TGGCGCTTTACTACTAATTTTTATTCTACTACTATTAGCACTA
P.coronatum    AGGCGCCCTCCTACTAATTTTTGCCTTATTATTAGTAGCTCTA
P.ditmarsi     AGGCGCACTACTACTAATTACTTTTCTTCTACTACTAGCACTA
P.douglasii    AGGTGCCCTACTATTAATTATTATTCTTCTACTATTAGCACTG
P.hernandesi   AGGTGCCCTATTACTAATCATTATTCTTCTACTATTAGCACTA
P.mcallii      AGGTGCCCTATTATTAATCATTATTCTACTATTCCTACCACTA
P.modestum     AGGGGCTCTATTATTAATTATAATCTTACTACTACTAGCATTA
P.orbiculare   AGGTGCCATACTATTAATTATTGTCCTACTACTCCTAGCACTA
P.platyrhinos  AGGCGCCCTATTATTAGTTGTTCTTTTACTATTACTAGCATTA
P.solare       AGGTGCCTTATTACTTATCTTTTCTCTACTAACACTAGCATTA
P.taurus       TGGCGCCATACTATTAATTATTATCTTGCTATTATTAGCACTA
S.merriami     AGGACTCCTTCTTCTAATTTTTACTCTTCTCCTGCTTGCACTT
U.ornatus      AGGTGCCCTACTATTAATTATTATTTTACTCTTACTAGCCCTA
U.stansburiana AGGAGCACTCCTACTAATTCTTCTTCTCCTACTTTTAGCACTA
Ancestor       AGGAGCMYTACTACTAATTATTMTTCTACTAMYACTWGCACTA

C.draconoides  TTTTCACCAAATCTACTAGGAGACCCAGAAAACTTTTCACCAG
C.texanus      TTTTCCCCTAACCTATTAGGAGACCCAGAAAATTTTTCACCGG
H.maculata     TTTTCACCAAACCTATTAGGAGACCCAGAAAATTTCTCACCAG
P.asio         TTCTCCCCAAACCTACTAGGAGACCCAGAAAACTTTTCTCCAG
P.braconnieri  TTTTCTCCAAATTTACTAGGAGACCCAGAAAACTTCTCCCCAG
P.cornutum     TTTTCCCCAAATATATTAGGAGACCCAGAAAACTTCTCCCCAG
P.coronatum    TTTTTTCCAAACCTACTAGGAGACCCAGAAAACTTTACCCCAG
P.ditmarsi     TTTTCCCCAAACCTCCTAGGAGACCCAGAAAACTTCTCCCCAG
P.douglasii    TTTTCCCCAAACCTTCTAGGGGACCCAGAAAACTTCTCCCCAG
P.hernandesi   TTTTCCCCAAACCTTCTGGGGGACCCGGAAAACTTCTCCCCAG
P.mcallii      TTTTTTCCAAACTTACTAGGAGACCCAGATAACTTTATCCCTG
P.modestum     TTTTTCCCAAACCTCCTAGGAGACCCAGAAAACTTCTCCCCAG
P.orbiculare   CTTTTTCCAAACCTTTTAGGAGATCCAGAAAACTTCTCCCCGG
P.platyrhinos  TTTTTTCCAAACCTTCTAGGAGACCCAGAAAATTTTTCCCCAG
P.solare       TTCTTTCCAAACCTACTAGGCGACCCAGAAAACTTTTCTCCAG
P.taurus       TTTTTTCCAAACCTACTAGGAGACCCAGAAAACTTCTCCCCAG
S.merriami     TTTTCACCAAATTTATTAGGAGACCCAGAAAACTTTACCCCAG
U.ornatus      TTTTCACCAAACCTCTTAGGAGACCCAGAAAACTTTACACCAG
U.stansburiana TTTTCACCTAACCTGCTAGGAGACCCAGAGAACTTCTCACCCG
Ancestor       TTTTCACCAAACCTAYTAGGAGACCCAGAAAACTTTTCACCAG

C.draconoides  CAAATCCACTAGTAACCCCTCCACACATTAAACCAGAATGATA
C.texanus      CAAACCCACTAGTCACCCCACCACACATCAAACCAGAATGATA
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H.maculata      CAAACCCACTAGTAACCCCCCCTCATATCAAACCAGAATGATA
P.asio          CCAACCCATTAGTAACACCACCCCATATCAAACCAGAGTGGTA
P.braconnieri   CAAACCCCCTAGTAACACCACCACACATTAAACCAGAATGATA
P.cornutum      CAAACCCACTAGTTACACCCCCACATATTAAACCAGAATGATA
P.coronatum     CAAACCCATTAGTAACACCACCCCACATCAAGCCAGAATGATA
P.ditmarsi      CAAACCCATTAGTAACACCCCCCCACATTAAACCAGAATGATA
P.douglasii     CCAACCCATTAGTAACACCACCTCATATTAAACCAGAATGATA
P.hernandesi    CCAACCCACTAGTAACACCCCCACACATTAAACCAGAATGATA
P.mcallii       CAGTTAA???????????????????????????????????
P.modestum      CCAATCCACTAGTAACACCCCCACATATCAAACCAGAATGATA
P.orbiculare    CTAACCCATTAGTAACCCCACCACACATTAAACCAGAGTGATA
P.platyrhinos   CTAATCCACTAGTAACACCCCCACACATTAAACCAGAATGATA
P.solare        CCAACCCACTAGTAACACCCCCACACATTAAACCAGAATGATA
P.taurus        CAAACCCCCTAGTAACACCCCCACATATCAAACCTGAGTGATA
S.merriami      CCAACCCCCTAATTACCCCACCCCACATTAAACCGGAATGATA
U.ornatus       CTAACCCCCTTGTTACACCACCACACATTAAACCAGAATGATA
U.stansburiana CCAACCCCCTAGTAACACCACCACATATTAAACCAGAGTGGTA
Ancestor        CMAACCCMCTAGTAACMCCACCACACATTAAACCAGAATGATA

C.draconoides   TTTCCTATTTGCCTACGCCATCCTACGATCAATTCCAAACAAA
C.texanus       CTTCCTATTTGCCTACGCCATCCTACGATCCATTCCAAACAAA
H.maculata      CTTCTTATTCGCCTATGCCATCTTACGATCTATTCCAAACAAA
P.asio          TTTTCTATTTGCCTACGCCATCCTACGATCCATTCCAAACAAA
P.braconnieri   CTTCCTATTTGCATATGCCATTTTACGATCAATTCCAAACAAA
P.cornutum      TTTCTTATTTGCCTACGCCATCTTACGATCAATCCCCAACAAA
P.coronatum     TTTTTTATTTGCCTATGCCATTCTACGATCAATTCCTAACAAG
P.ditmarsi      CTTCTTATTTGCCTATGCTATCCTACGATCAATCCCCAACAAG
P.douglasii     CTTTCTATTTGCCTATGCTATTTTACGATCCATTCCTAACAAA
P.hernandesi    TTTTTTATTTGCCTATGCTATTTTACGATCAATCCCCAACAAA
P.mcallii       ????????????????????????????????????????
P.modestum      TTTCTTATTTGCTTATGCCATTTTACGATCAATCCCAAACAAA
P.orbiculare    CTTTCTATTTGCCTATGCCATCTTACGATCAATTCCAAACAAA
P.platyrhinos   TTTTCTATTTGCCTACGCTATTTTACGATCAATTCCAAACAAA
P.solare        CTTCCTATTTGCTTATGCTATTCTACGATCAATCCCCAACAAA
P.taurus        TTTTCTATTTGCATACGCTATCTTACGATCAATTCCAAACAAA
S.merriami      TTTCCTATTTGCATACGCTATTCTCCGATCTATCCCAAACAAA
U.ornatus       TTTCCTATTCGCCTATGCTATCCTACGATCAATTCCAAACAAA
U.stansburiana CTTTTTATTTGCCTACGCCATTCTACGATCAATTCCAAATAAA
Ancestor        YTTCCTATTTGCCTACGCCATYCTACGATCAATTCCAAACAAA

C.draconoides   TTGGGAGGTGTACTCGCCTTACTTTTTTCAATCCTCATCCTCA
C.texanus       CTGGGGGGAGTACTAGCCTTACTCTTTTCAATCCTAATCCTAA
H.maculata      CTAGGGGGCGTACTTGCCCTTCTTTTCTCAATTTTGATTCTCA
P.asio          CTGGGGGGAGTTTTAGCCCTATTATTTTCCATCTTAATCCTAA
P.braconnieri   CTGGGTGGAGTTCTTGCCCTATTATTCTCAATTCTAGTCTTAA
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P.cornutum      CTAGGAGGTGTTTTAGCCCTACTATTTTCCATCATAGTCCTTA
P.coronatum     TTAGGAGGCGTCCTTGCTCTACTATTCTCAATTTTAATCCTAA
P.ditmarsi      CTAGGCGGTGTCCTTGCCCTCCTATTCTCAATCTTAATCTTAA
P.douglasii     TTAGGTGGCGTTCTTGCCCTACTATTCTCAATCTTAATCTTAA
P.hernandesi    CTAGGTGGTGTCCTTGCCCTCCTATTTTCAATCCTAATCTTAC
P.mcallii       ??????????????????????????????????????????
P.modestum      CTAGGCGGAGTCCTTGCCCTACTATTATCAATCTTAATCCTAA
P.orbiculare    CTTGGTGGTGTCCTTGCCCTACTATTTTCAATTTTAGTTTTAA
P.platyrhinos   TTAGGGGGGGTCCTCGCCTTATTATTCTCAATTTTAATCTTAA
P.solare        CTAGGAGGAGTCCTTGCCCTACTTTTCTCAATCCTAGTCCTAA
P.taurus        CTAGGCGGAGTCCTCGCCTTATTATTTTCAATTCTAGTCCTAA
S.merriami      CTCGGCGGCGTACTAGCCCTATTATTTTCTGTCTTAATCTTAA
U.ornatus       CTAGGAGGAGTTCTAGCCCTACTATTTTCAATTTTAATCCTCA
U.stansburiana  CTAGGAGGAGTACTTGCCCTACTTTTCTCAATCATAATCTTAA
Ancestor        CTRGGAGGAGTACTWGCCYTACTTTTTTCAATCHTAATCYTAA

C.draconoides   TACTAGTCCCAATAATACACACATCAAAACAACGAAGCACCTC
C.texanus       TACTAGTTCCACTACTACATACATCAAAACAACGAAGCGCCTC
H.maculata      TATTAGTTCCTTTACTACACACATCAAAACAACGAAGCACCTC
P.asio          TGCTAATTCCACTACTACACACATCAAAACAACGAAGCATTAT
P.braconnieri   CACTAACCCCACTTCTACATACATCAAAACAACGAAGCACTTC
P.cornutum      TACTAATCCCACTTCTACACACATCAAAACAACGCAGCTCCTC
P.coronatum     TGTTAATTCCACTATTACACACATCAAAACAACGAAGTAACTC
P.ditmarsi      TGCTTATCCCACTCCTACACACATCAAAACAACGAAGCGCTTC
P.douglasii     TACTTATCCCACTCCTACATACATCAAAACAACGAAGTACTTC
P.hernandesi    TGCTTATTCCCCTCCTACACATATCAAAACAACGAAGTACTTC
P.mcallii       ??????????????????????????????????????????
P.modestum      TACTCGTTCCACTCTTACATACATCAAAACAACGAGGCACTTC
P.orbiculare    TACTTATTCCGCTCCTACACACATCGAAACAACGAAGTACCTC
P.platyrhinos   TATTAATTCCACTTCTACACATATCAAAACAACGGACCACTTC
P.solare        TATTTATTCCACTACTACATACATCAAAACAACGAAGCAATTA
P.taurus        TATTAATTCCACTTCTACATACATCAAAACAACGAAGTACCTC
S.merriami      CACTAGTCCCCCTCCTCCACACCTCAAAACAACGTAGTACAAT
U.ornatus       TACTAGTGCCACTCTTACACACATCTAAACAACGAAGCACTAT
U.stansburiana  TAATTGTTCCACTACTTCATACATCAAAACAACGAAGCACAAC
Ancestor        TACTAGTYCCACTACTACACACATCAAAACAACGAAGCACCWC

C.draconoides   CTTCCGACCAATATCTCAAACCATATTTTGACTTTTAATCTCA
C.texanus       ATTCCGCCCAATATCTCAGATTATATTCTGATTTCTAATTGCA
H.maculata      CTTCCGTCCAGCCTCCCAAACTATATTTTGACTTTTAATTTCA
P.asio          ATTTCGACCAATCTCACAAACCATGTTTTGACTACTAATCTCA
P.braconnieri   TTTCCGCCCAATTTCACAAATTATATTTTGACTATTAATTTCA
P.cornutum      CTTTCGACCAATATCACAAATTATATTCTGACTATTAATCTCA
P.coronatum     CTTCCGACCAATCTCACAAATTATATTCTGATTATTAATTTCA
P.ditmarsi      CTTCCGACCAATTTCACAAAGCATATTTTGACTCCTAACTTCA
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P.douglasii     ATTCCGACCAATTTCACAAAGCATATTTTGACTTTTAATTTCA
P.hernandesi    CTTCCGACCAATTTCACAAAGCATATTTTGACTCTTAATTTCA
P.mcallii       ????????????????????????????????????????
P.modestum      CTTCCGACCAATTTCACAAACCATATTCTGACTATTAATTTCA
P.orbiculare    ATTCCGACCAATCTCACAAACCATGTTCTGACTACTAATTTCA
P.platyrhinos   ATTCCGACCCATCTCACAAACGATGTTCTGACTATTAATTTCA
P.solare        CTTCCGACCAGTATCACAAACCATATTCTGACTATTAGTTTCA
P.taurus        TTTCCGACCAATTTCACAAATTATATTCTGACTACTAATTTCA
S.merriami      ATTTCGTCCTACATCACAGATAATATTTTGACTACTAATTTCA
U.ornatus       ATTCCGCCCAATATCTCAAATTATATTTTGACTCCTAATTTCA
U.stansburiana  ATTCCGCCCAATCTCCCAACTTATATTCTGACTTTTAATCTCA
Ancestor        MTTCCGCCCAATATCWCAAAYTATATTTTGACTTTTAATYTCA

C.draconoides   GATGTCCTCATTCTTACATGAATTGGGGGACAACCTGTA?AAC
C.texanus       GACGTACTTATCCTAACATGAATTGGAGGTCAACCAGTAGAAC
H.maculata      AACGTACTTATCCTCACATGAATTGGAGGACAACCTGTAGAAC
P.asio          AACGTACTAGTACTAACATGAATTGGAGGACAACCAGTAGAAC
P.braconnieri   GACGTATTTATTTTAACATGAATTGGAGCTCAACCAGTTGAAC
P.cornutum      GACGTATTTATTCTAACATGAATTGGAGGACAGCCCGTCGAAC
P.coronatum     AACATATTTATTCTAACATGAATTGGAGGCCAACCAGTCGAAC
P.ditmarsi      GACCTTCTTATCTTAACATGAATTGGGGGCCAACCAGTTGAAC
P.douglasii     GATATATTTATTTTAACATGAATTGGAGGTCAACCAGTTGAAC
P.hernandesi    GACCTTCTTATCCTAACATGGATTGGAGGTCAACCCGTTGAAC
P.mcallii       ????????????????????????????????????????
P.modestum      AACGTACTAATCTTAACATGAATCGGAAATCAACCAGTTGAAC
P.orbiculare    GATGTATTTATTTTAACCTGAATTGGAGGCCAACCGGTTGAAC
P.platyrhinos   GATGTAATTATTCTGACATGAATTGGAGGACAACCAGTTGAAC
P.solare        GACGTATTTATCTTAACCTGAATCGGGGGCCAACCAGTTGAAC
P.taurus        GACGTACTTATCTTAACATGAATTGGAGGCCAACCAGTTGAAC
S.merriami      GATGTACTAATCCTAACATGAATCGGAGGTCAACCTGTAGAAC
U.ornatus       GACGTACTCATTCTAACATGAATCGGAGGTCAACCAGTTGAAC
U.stansburiana  GACGTACTAATCCTAACATGAATCGGAGGACAGCCAGTAGAGC
Ancestor        GACGTACTWATCCTAACATGAATYGGAGGACAACCAGTAGAAC

C.draconoides   ACCCATTTATCATTATTGGACAACTTGCCTCAATCACTTACTT
C.texanus       ACCCATTCATTATTATTGGCCAACTTGCCTCAATTATTTATTT
H.maculata      ACCCATTTATTATCATTGGGCAACTTGCCTCAACAACCTACTT
P.asio          ATCCATTCACTATTATTGGTCAATTAGCATCAATCACCTACTT
P.braconnieri   ACCCATTTATTATTATCGGACAACTTGCCTCAATTCTCTACTT
P.cornutum      ACCCATTTATTATTATTGGACAACTTGCCTCAATTATTTACTT
P.coronatum     ACCCATTTATTATTATTGGCCAACTCGCCTCAATCACCTACTT
P.ditmarsi      ACCCATTTATCATTATTGGACAACTTGCTTCAATAACCTACTT
P.douglasii     ACCCATTTATTATTATTGGACAACTTGCCTCAGTAATTTACTT
P.hernandesi    ATCCATTTATTATCATTGGACAACTTGCCTCAGTAATCTACTT
P.mcallii       ????????????????????????????????????????
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P.modestum      ACCCATTTATTATTATCGGACAACTCGCCTCAATTTCTTATTT
P.orbiculare    ACCCATTTATTATCATCGGACAGCTTGCCTCAGTGATCTATTT
P.platyrhinos   ACCCATTTATTATTATTGGTCAACTCGCCTCAATTATTTACTT
P.solare        ACCCATTTATTATCATTGGCCAACTCGCTTCAGTAGCCTATTT
P.taurus        ACCCATTTATTATTATTGGTCAACTTGCCTCAATCATCTACTT
S.merriami      ACCCATTCATTGTCATCGGCCAACTTGCCTCAATTATCTACTT
U.ornatus       ACCCATTTATCATCATTGGTCAATTAGCCTCAATTATATACTT
U.stansburiana  ACCCATTCATTATTATCGGACAACTAGCCTCAATTATTTATTT
Ancestor        ACCCATTYATTATTATYGGMCAACTWGCCTCAATTAYTTACTT

C.draconoides   CCTTCTATTCTTTATTTTTATACCACCACCAGC?ATCCTAGAG
C.texanus       TTTTCTATTTCTACTTATTATACCAACAACAGCAATGCTAGAA
H.maculata      CTTATTATTTTTATTTTTTATACCAATAACAGCAATACTAGAA
P.asio          CCTACTATTCCTAATCATCATACCAACAACAGCAATCCTAGAA
P.braconnieri   CTTTTTATTTTTAATCCTTTTACCAACAATAGCCACCCTAGAA
P.cornutum      TCTATTATTTTTAATCCTTATACCAACCATATCCACACTAGAA
P.coronatum     TTCACTATTCCTAATCCTTATACCAATCACAGCAATCCTAGAA
P.ditmarsi      CTCCTTATTCTTAATCCTAATCCCAATTACAGCCGCCCTAAAA
P.douglasii     CTCATTATTCTTAATTCTTATACCAACTATAGCCACTCTAGAA
P.hernandesi    CTCACTATTCTTAATTCTTATGCCAATTTTAGCCACCATAGAA
P.mcallii       ??????????????????????????????????????????
P.modestum      TTTATTATTTTTAATTCTAATGCCAACTATAGCTATTCTAGAA
P.orbiculare    CTCATTATTCTTAATCCTTATGCCAACTATAGCCACCATAGAA
P.platyrhinos   CTCATTATTTTTAATCCTCATACCAACTACCGCCATACTAAAA
P.solare        CATACTATTTTTAATTATTATACCAACCACAGCCATCCTAGAA
P.taurus        CTCTCTATTTTTAATTCTACTTCCAACCACAGCTACCCTAGAA
S.merriami      TATACTTTTCTTAATCCTTATACCATACACCGCCATATTAGAA
U.ornatus       TACACTATTTCTTGTTCTCATACCAATAACATCTATTCTAGAA
U.stansburiana  TATATTATTCCTGGTCCTAATACCAATCATTTCATCCCTAGAA
Ancestor        YHTWCTATTCYTARTYYTTATACCAAYMACAKCAATMCTAGAA

C.draconoides   AACAAACTCCTAAAATGATAATTAAAAATGGGAGGATATGGAA
C.texanus       AACAAACTACTAAAATGATAACTAAAAATAGGGGGCTATGGGA
H.maculata      AACAAGCTTCTAAAATGGTAATTAAAAATAGGAGGGTATGGAA
P.asio          AACAAATTACTAAAATGATAA--------------CTACGGAA
P.braconnieri   AACAAACTATTAAAATGATAA------------------GGAA
P.cornutum      AACAAACTATTAAAATGATAGCTAAAAATGGGAGGATATGGAA
P.coronatum     AACAAACTATTAAAATGGTAA----------------------
P.ditmarsi      AACAAACTACTAAAATGATAATTAAAAATAGGTGGCTATGGAA
P.douglasii     AACAAACTACT?AAATGATAATTAAAAATAGGTGGCTATGGAA
P.hernandesi    AACAAACTACTAAAATGATAATTAAAAATAGGGGGCTATGGAA
P.mcallii       ??????????????????????????????????????????
P.modestum      AACAAACTATTAAAATGATAACTAAAAATGGGGGGTTATGGTA
P.orbiculare    AATAAACTATTAAAATGATAATTAAAAATAGGAGGCTATGGGA
P.platyrhinos   AACAAACTATTAAAATGATAGCTAAAAATAGGAGGTTATGGTA
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P.solare        AATAAACTATTAAAATGATAGTTAAAAATAGGAGGCTATGGTA
P.taurus        AATAAACTATTCTAATGATAATTAAAAATAGGAGGCTATGGAA
S.merriami      AACAAACTTCTCAAATGATAACTAAAACTCGGAGGCTACGGTA
U.ornatus       AATAAACTACTCAAATGATAGCTAAAATTAGGAGGATATGGAA
U.stansburiana AATAAACTTTTAAAATGATAACTAAAACTAGGAGGATATGGCA
Ancestor        AAYAAACTWCTAAAATGATAAYTAAAAATAGGAGGATATGGAA

C.draconoides   TTATTCGGATTACAATGACTTTAATACCCATAACACCAAAATT
C.texanus       TTATTCGAATCACAATAGCCCTCTCACCACTAACACCAAAGTT
H.maculata      TTATCCGAGTTACTATGATCCTAACACCACTAACACCAAAACT
P.asio          TTATACGAATTACAACCACACTAGCCCCACTAACTCCAAAACT
P.braconnieri   TCATCCGAATTACCATAATATTAACCCCCCTAACCCCAAAACT
P.cornutum      TTATCCGAATCACCACGATACTGTCACCACTAACCACAAAACT
P.coronatum     ----------------------------------CAAAACT
P.ditmarsi      TTATCCGAATCACCATAACATTAATACCCCTCACCCCAAAACT
P.douglasii     TTATCCGAATCACTATAACACTAACACCCCTTACTTCAAAACT
P.hernandesi    TTATCCGAATTACTATAGCACTAGCACCCCTTACCCCAAAACT
P.mcallii       ????????????????????CACTATCCCCGCTAACTCCAAAACT
P.modestum      TTATCCGAATTACTATAACACTAACACCACTTACCCCAAAACT
P.orbiculare    TTATCCGAATCACTATAACATTAATACCACTTACTCCAAAACT
P.platyrhinos   TTATTCGAATTACCATAACACTATCCCCATTAACTCCAAAACT
P.solare        TTATTCGAATTACCATAGCACTATCCCCACTAACCCCCAAACT
P.taurus        TTATCCGAATTACCATAACATTAACCCCCCTAACCCCAAAACT
S.merriami      TTATACGAATTACTTTATCCCTTGCTCCACTAACCCCCAAACT
U.ornatus       TCATTCGAATTACAATAACACTAACCCCACTAACA?CAAAACT
U.stansburiana TCATTCGAATCACAATATCACTTACCCCGCTCACCCCAAAACT
Ancestor        TYATTCGAATTACAATRACACTAACMCCACTAACMCCAAAACT

C.draconoides   ATACTATCCTTTTATAATCTTAGCCCTTTGAGGCATTGTAATA
C.texanus       CTACTACCCATTTATAATCTTGGCCCTTTGAGGAATTGTTATG
H.maculata      ATATTACCCCTTCATAATTCTAGCCCTCTGAGGCATTGTAATA
P.asio          CTACTACCCATTTATAATCCTCGCACTATGAGGCATTATTATA
P.braconnieri   TTATTACCCCTTTATAATCCTCGCACTCTGAGGCATTATTATA
P.cornutum      CTATTACCCATTTTTAATTCTTGCACTATGAGGCATCATTATA
P.coronatum     TTACTACCCGTTCATTATCCTTGCACTATGAGGCATTATTATA
P.ditmarsi      CTACTACCCGTTTATAATCCTTGCACTATGAGGCATTATTATA
P.douglasii     CTACTACCCATTTATAATCCTCGCACTATGGGGTATTATTATG
P.hernandesi    CTACTACCCATTTATAATCCTCGCACTATGAGGCATTATTATA
P.mcallii       CTATTATCCATTTATTATTCTTGCACTATGAGGCATTATTATA
P.modestum      CTATTATCCATTCATAATCCTAGCACTATGAGGTATCATTATA
P.orbiculare    TTATTATCCATTTATAATCCTTGCACTTTGGGGTATTATTATA
P.platyrhinos   CTACTACCCGTTTATTATTCTTGCACTATGGGGTATTATTATA
P.solare        CTATTACCCCTTTATTGTTCTCGCACTATGGGGCATTATTATA
P.taurus        CTATTACCCATTTATAATCCTCGCACTTTGAGGTATCATCATA
S.merriami      CTATTACCCATTTTTAATCTTAGCATTATGAGGAATTGTAATA
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U.ornatus       ATGCTACCCATTCATAATCCTTGCTCTATGGGGAATCGTCATA
U.stansburiana TTGTTACCCATTTATAATCCTTGCCCTATGAGGCATCGTAATA
Ancestor        HTAYTACCCATTYATAATCCTWGCMCTHTGAGGCATYGTMATA

C.draconoides   ACCAGTTCAATCTGCATACGACAAACAGACCTAAAATCCTTAA
C.texanus       GCCAGCTCAATTTGTATACGACAAACAGACCTAAAGTCCATAA
H.maculata      ACCAGCTCCATTTGCATACGTCAAACAGACCTAAAATCCCTAA
P.asio          ACCAGCTCAATCTGCCTTCGCCAAACAGACCTAAAATCATTAA
P.braconnieri   GCTAGCTCAATTTGCCTACGTCAAACAGACCTAAAATCACTAA
P.cornutum      ACAAGCTCAATCTGTATGCGCCAAACCGACCTAAAATCATTAA
P.coronatum     ACTAGCTCAATTTGCTTACGTCAAACAGACCTTAAATCGTTAA
P.ditmarsi      ACGAGCTCGATCTGCTTACGTCAAACAGACCTAAAATCACTAA
P.douglasii     ACTAGCTCAATCTGCCTACGTCAAACAGATCTTAAATCACTAA
P.hernandesi    ACTAGCTCAATTTGCTTACGTCAGACAGACCTAAAATCACTAA
P.mcallii       ACTAGCTCAATTTGTTTACGCCAAACAGACCTCAAATCACTAA
P.modestum      ACTAGCTCAATTTGTATACGTCAAACAGACCTTAAATCATTAA
P.orbiculare    ACTAGCTCAATTTGTTTACGTCAAACAGACCTTAAATCAATAA
P.platyrhinos   ACTAGCTCAATCTGCTTACGTCAAACAGACCTTAAATCATTAA
P.solare        AGTAGCTCAATTTGCTTACGTCAAACAGATCTAAAGTCGTTAA
P.taurus        ACTAGCTCAATCTGTTTACGTCAAACAGACCTAAAATCATTAA
S.merriami      ACAAGCTCTATCTGCATACGACAAACAGACCTAAAATCCATAA
U.ornatus       ACTAGTTCAATTTGCATACGACAAACCGACCTAAAATCCATAA
U.stansburiana ACCAGTTCAATCTGCCTACGACAAACAGACCTAAAATCTCTAA
Ancestor        ACCAGCTCAATTTGCATACGWCAAACAGACCTAAAATCCATAA

C.draconoides   TTGCCTATTCATCAGTAAGCCATATAGGCCTTGTAGTAGCAGC
C.texanus       TCGCTTACTCATCAGTAAGTCACATGGGACTAGTAGTAGCAGC
H.maculata      TTGCCTACTCATCAGTAAGCCACATAGGCCTCGTAATTGCATC
P.asio          TCGCTTACTCATCTGTTAGCCACATGGGCCTTGTAGTAATAGC
P.braconnieri   TTGCTTACTCCTCTGTCAGCCACATAGGCCTTGTAGTAATAGC
P.cornutum      TCGCATACTCCTCTGTTAGCCACATAGGCCTTGTAGTAACAGC
P.coronatum     TCGCCTACTCCTCCGTTAGTCATATAGGCCTTGTAGTAATAGC
P.ditmarsi      TCGCCTATTCCTCCGTTAGCCATATAGGCCTTGTAGTAATAGC
P.douglasii     TTGCTTATTCCTCTGTTAGCCACATAGGCCTTGTAGTAATAGC
P.hernandesi    TTGCCTATTCCTCTATCAGCCATATAGGTCTTGTAGTAATAGC
P.mcallii       TCGCCTACTCTTCAGTCAGTCATATGGGGCTTGTAGTAATAGC
P.modestum      TTGCTTATTCCTCTGTAAGCCACATGGGCCTTGTAGTAATAGC
P.orbiculare    TCGCCTATTCATCCATCAGCCATATAGGCCTTGTAGTAATAGC
P.platyrhinos   TTGCCTATTCCTCAGTTAGCCATATAGGACTTGTAGTAATAGC
P.solare        TCGCCTACTCCTCTATTAGCCACATAGGCCTTGTAGTAATAGC
P.taurus        TTGCCTATTCCTCTGTTAGCCACATGGGCCTTGTAGTAATAGC
S.merriami      TCGCCTATTCATCAGTCAGCCATATAGGACTTGTTATTACAGC
U.ornatus       TCGCCTACTCATCAGTAAGTCATATAGGCCTAGTAATCGCAGC
U.stansburiana TCGCCTACTCATCCGTTAGCCACATAGGACTCGTCATTACAGC
Ancestor        TYGCCTACTCATCAGTAAGCCAYATAGGCCTTGTARTWRCAGC
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C.draconoides  TTGCTTAATTCAGACACCATGAAGCTTCACAGGAGCCATAATC
C.texanus      CTGCCTGATCCAAACACCATGAAGTCTTACAGGTGCTATAATC
H.maculata     TTGTTTAATTCAAACACCATGAAGCTTTACAGGCGCTATAATT
P.asio         ATGCCTAATACAAACACCATGAAGCTTCACAGGAGCCATAATA
P.braconnieri  CTGCCTAATCCAAACCCCATGAAGCATTTCAGGAGCTATAATC
P.cornutum     CTGCCTAATTCAAACACCGTGAAGCCTCACAGGAGCCATAATC
P.coronatum    ATGTTTAATCCAAACACCATGAAGCTTCACAGGAGCTATAATC
P.ditmarsi     CTGCCTAATCCAAACACCATGAAGCTTTACAGGAGCTATTGTA
P.douglasii    CTGCCTGATCCAAACACCATGAAGCTTCACAGGAGCTATTATG
P.hernandesi   CTGCCTAATCCAAACACCATGAAGCTTTACAGGAGCTATTATA
P.mcallii      TTGCTTAATTCAAACACCATGAAGCTTCACAGGAGCTATTGTT
P.modestum     TTGCCTTATTCAAACACCATGAAGCTTCACAGGCGCTATAATA
P.orbiculare   ATGCCTAATCCAAACACCATGAAGTTTCACAGGAGCTATTATA
P.platyrhinos  TTGTCTAATCCAAACACCATGAAGCTTCACAGGAGCTATAATA
P.solare       ATGCCTTATCCAAACACCATGAAGCTTCACAGGAGCCATAATC
P.taurus       CTGCCTAATCCAAACCCCATGAAGCTTCACAGGAGCTATAATC
S.merriami     CTGCCTAATTCAAACACCATGAAGTTTTACAGGAGCTATAATC
U.ornatus      ATGCCTAATCCAAACACCATGAAGCTTCACCGGAGCCATAATC
U.stansburiana CTGCCTAATTCAAACACCATGAAGCTTTACTGGGGCCATAATA
Ancestor       HTGCYTAATTCAAACACCATGAAGCTTTACAGGAGCYATAATC

C.draconoides  CTAATAATTGCTCATGGATTAACATCATCCATACTATTCTGTC
C.texanus      CTAATAATTGCACACGGATTAACATCATCAATACTATTCTGCC
H.maculata     TTAATAATCGCACATGGACTAACTTCATCCATACTATTCTGTT
P.asio         CTAATAATTGCACACGGACTAACATCCTCCATACTATTCTGCC
P.braconnieri  CTAATAATTGCACACGGACTAACCTCTTCCATACTATTCTGCC
P.cornutum     TTAATAATTGCACACGGACTAACTTCATCCATATTATTCTGTT
P.coronatum    CTAATAATTGCACACGGACTAACCTCCTCCATATTGTTCTGCT
P.ditmarsi     TTAATAGTTGCACATGGACTAACCTCCTCCATACTATTCTGTT
P.douglasii    CTAATAATTGCACATGGACTAACCTCATCCATATTATTCTGTT
P.hernandesi   TTAATAATTGCACATGGGCTAACCTCCTCCATACTATTCTGTT
P.mcallii      TTAATAATTGCACATGGATTAACCTCATCCATATTATTTTGCC
P.modestum     TTAATAATTGCACATGGACTAACCTCATCCATACTATTCTGCT
P.orbiculare   TTAATAATTGCACATGGCCTAACCTCCTCTATACAATTCTGCC
P.platyrhinos  TTAATAATTGCACACGGACTAACCTCCTCCATATTATTCTGCC
P.solare       TTAATAATCGCACATGGATTAACCTCATCCATACTATTCTGTT
P.taurus       TTAATAATCGCACACGGATTAACCTCATCCATATTATTCTGTC
S.merriami     TTAATAATCGCACATGGCTTAACTTCATCTATACTATTCTGTT
U.ornatus      CTGATAATTGCACACGGACTAACCTCATCAATACTTTTTTGCT
U.stansburiana TTAATAATTGCACACGGCCTCACTTCATCCATACTATTCTGCC
Ancestor       YTAATAATTGCACAYGGACTAACTTCATCCATACTATTCTGYT

C.draconoides  TAGCAAATACAAACTATGAACGAACTCACAGCCGAACAATAAT
C.texanus      TAGCAAACACAAACTATGAACGAACCCACAGCCGAACAATAAT
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H.maculata      TAGCAAATACAAACTATGAACGAACCCACAGCCGAACAATAAT
P.asio          TAGCAAACACAAATTATGAACGAACTCACAGCCGAACACTAAT
P.braconnieri   TAGCAAATACAAATTATGAACGAACCCATAGCCGAACATTAAT
P.cornutum      TAGCAAACACAAACTATGAACGAACCCACAGTCGAACATTAAT
P.coronatum     TAGCAAACACAAACTATGAACGAACCCACAGCCGAACATTAAT
P.ditmarsi      TAGCAAATACAAATTACGAGCGAACACACAGCCGAACCCTGAT
P.douglasii     TAGCAAATACAAACTATGAACGAACACACAGCCGAACCCTAAT
P.hernandesi    TAGCAAATACAAATTATGAACGAATACACAGCCGAACCTTAAT
P.mcallii       TAGCAAACACAAACTATGAACGAACTCATAGCCGAACCCTAAT
P.modestum      TAGCAAACACAAACTACGAACGAACCCACAGCCGAACAATAAT
P.orbiculare    TAGCAAACACAAATTATGAACGAACTAATAGCCGAACATTAAT
P.platyrhinos   TAGCAAACACGAACTACGAACGAACTCATAGCCGAACACTAAT
P.solare        TAGCAAACACAAACTATGAACGAACCCATAGCCGAACACTAAT
P.taurus        TAGCAAATACAAATTATGAACGAACTCACAGCCGAACACTAAT
S.merriami      TAGCAAACACAAACTACGAACGAACCCACAGTCGAACTTTAAT
U.ornatus       TAGCAAATACAAACTATGAACGAACCCACAGTCGAACCCTAAT
U.stansburiana  TAGCAAATACAAACTACGAACGAACCCACAGTCGAACCCTAAT
Ancestor        TAGCAAATACAAACTATGAACGAACCCACAGYCGAACMMTAAT

C.draconoides   TTTAGCACGCGGTTTACAACTCATTCTTCCCATTATGGCAACT
C.texanus       CTTAGCACGAGGTTTACAACTAATTCTTCCAATAATAACAACT
H.maculata      TTTAGCACGAGGCCTACAACTAATTCTTCCAATCATAGCAACT
P.asio          TCTAGCCCAAGGACTTCAACTGGTTTTACCACTAATAATGACC
P.braconnieri   CCTAGCCCGCGGTCTTCAACTAATCCTTCCACTAATAATGACC
P.cornutum      TTTAGCCCGAGGATTTCAACTAATTTTTCCACTAATAACAACC
P.coronatum     TCTAGCCCAAGGACTTCAACTAATTTTTCCACTAATAACAACC
P.ditmarsi      TTTAGCCCGAGGCCTACAACTAATCCTCCCATTAATAATAGCT
P.douglasii     CCTGGCCCGAAGCCTACAATTAATCCTTCCATTAATAATGGCC
P.hernandesi    TTTAGCCCGAGGCCTACAACTAATTCTTCCATTAATAATAACT
P.mcallii       TTTAGCCCGAGGCCTCCAACTTATTTTTCCACTAATAACAACC
P.modestum      CCTGGCCCGAGGCCTTCAACTTATCTTTCCATTAATAATAACC
P.orbiculare    CCTAGCCCGAGGCCTTCAACTAATTCTCCCACTAATAATAACT
P.platyrhinos   TTTAGCCCGAGGCCTTCAACTTATTTTTCCATTAATAACAACC
P.solare        TCTTGCCCGCGGCCTTCAACTCATTCTCCCACTAATAATAACC
P.taurus        TTTAGCCCGAGGCCTTCAACTAATTCTTCCACTGATAATGCTC
S.merriami      GCTTGCCCGCGGATTCCAACTTATTCTCCCACTAATATCAACC
U.ornatus       TCTTGCTCGAGGACTACAACTTATTCTTCCACTAATAACCACC
U.stansburiana  TCTCGCCCGAGGCTTTCAAATAATCCTACCACTAATAACAACT
Ancestor        TYTWGCCCGAGGHYTACAACTWATTCTTCCACTAATAACAACY

C.draconoides   TGATGACTGCTAGCAAACCTAACTAACATAGCGCTACCCCCAT
C.texanus       TGATGATTACTAGCAAACCTCACCAACATAGCACTACCCCCAT
H.maculata      TGATGACTTCTAGCAAACTTAACCAATATAGCACTCCCACCAT
P.asio          TGATGACTTCTAGCTAACCTAACTAACATAGCACTTCCCCCAT
P.braconnieri   TGATGACTTCTAGCAAACCTAACCAACATAGCACTTCCCCCAT
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P.cornutum      TGATGACTACTGGCTAACCTAACCAACATAGCACTTCCCCCAT
P.coronatum     TGATGACTCTTAGCAAACATAACCAACATAGCACTCCCCCCAA
P.ditmarsi      TGATGACTTCTAGCTAACCTAACAAACATAGCCCTACCCCCCT
P.douglasii     TGATGGCTTCTAGCAAACCTAACAAACATAGCACTTCCCCCAT
P.hernandesi    TGATGACTTCTAGCAAACCTGACAAACATAGCACTTCCCCCAT
P.mcallii       TGATGACTTCTAGCAAACCTCACCAACATAGCACTTCCCCCCT
P.modestum      TGATGACTTTTAGCAAACCTAACAAACATAGCACTTCCACCAT
P.orbiculare    TGATGACTTCTAGCAAACCTAACAAACATAGCACTTCCCCCAT
P.platyrhinos   TGATGACTTCTAGCAAACCTAACCAACATAGCACTTCCCCCCT
P.solare        TGATGACTCTTAGCAAACCTAACAAACATAGCACTTCCACCAT
P.taurus        TGATGACTTTTAGCAAATCTAACTAATATAGCACTTCCCCCAT
S.merriami      TGATGACTACTAGCAAACCTAACAAACATAGCACTACCACCAT
U.ornatus       TGATGACTTCTAGCAAACCTTACTAATATGGCACTACCACCAT
U.stansburiana TGATGGCTTCTAGCAAACTTAACCAACATAGCACTACCACCAT
Ancestor        TGATGACTTCTAGCAAACCTAACCAAYATAGCACTACCACCAT

C.draconoides   CAATTAATCTAATAGGAGAACTATTAATTATTGTCTCCCTATT
C.texanus       CAATCAACCTTATAGGAGAACTATTTATTATTACTTCTCTGTT
H.maculata      CAATTAACCTAATAGGCGAACTATTAATTA?TATTTCCCTATT
P.asio          CAATTAACCTAATAGGAGAACTATTAATCATCGTATCACTATT
P.braconnieri   CAATCAATCTAATAGGAGAACTACTCATCATTATTTCACTCTT
P.cornutum      CAATCAACCTAATAGGAGAACTATTTATTATCGTTTCACTATT
P.coronatum     CAATTAATTTAATAGGAGAACTATTTATCATTGTATCACTATT
P.ditmarsi      CAATTAATCTGATGGGCGAGTTATTTATTATTGTATCATTATT
P.douglasii     CAATTAATCTAATAGGAGAATTATTTATTATTGTGTCATTATT
P.hernandesi    CAATTAATCTGATAGGAGAATTATTTATTATTGTATCATTGTT
P.mcallii       CAATTAATTTTATAGGAGAATTATTTATCATTTTATCACTATT
P.modestum      CAATTAATCTAATAGGAGAACTATTTATTATTGTATCACTATT
P.orbiculare    CAATTAACCTAATAGGAGAACTGTTCATCATTGTATCACTGTT
P.platyrhinos   CAATTAATCTTATAGGAGAACTATTTATTATTTTGTCACTATT
P.solare        CAATCAACCTAATAGGAGAACTTTTAATTATTGTATCATTATT
P.taurus        CAATTAACCTAATAGGAGAAATATTTATTATTATTTCACTATT
S.merriami      CAATTAATCTAATAGGAGAACTACTTATTATTACTTCACTATT
U.ornatus       CCATTAACCTAATAGGTGAATTACTCATTATCATTTCATTATT
U.stansburiana CAATCAATTTAATAGGAGAACTACTTATTATTACCTCACTATT
Ancestor        CAATTAACCTAATAGGAGAACTAYTHATTATTATTTCACTATT

C.draconoides   TAACTGATCTTCCCCTACAATTGTATTAACAGGATTAGGAACA
C.texanus       CAACTGATCCTGCCCAACAATCATTATAACTGGACTAGGAACC
H.maculata      CAACTGATCCCCCCCCAACAATTGTAATAACCGGACTAGGCACC
P.asio          CAACTGATCCTCACCAACTATTCTACTAACAGGACTGGGCACA
P.braconnieri   TAATTGATCTTTTCCAACAATCCTACTAACAGGACTAGGCACA
P.cornutum      TAACTGATCCTTCCCAACAATTTTACTAACAGGATTAGGCACA
P.coronatum     TAACTGATCATTTCCAACAATCTTACTAACTGGCCTAGGTACA
P.ditmarsi      TAACTGGTCTCTCCCAACAATTTTATTAACTGGCTTGGGCACA
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P.douglasii     TAACTGATCCCTCCCAACAATTCTACTAACTGGACTAGGTACA
P.hernandesi    CAACTGATCCCTCCCAACAATTCTATTAACTGGGTTAGGCACA
P.mcallii       TAACTGATCCTTCCCAACAATTTTATTAACCGGACCGGGCACA
P.modestum      CAACTGATCATTCCCAACAATTCTATTAACTGGATTAGGTACC
P.orbiculare    CAACTGATCCTTCCCAACAATTCTATTAACTGGACTAGGTACA
P.platyrhinos   TAACTGATCTTTCCCAACAATTTTATTAACAGGACTAGGTACA
P.solare        CAACTGATCCTTCCCAACAATTTTACTAACAGGACTCGGCACA
P.taurus        TAATTGATCTCTTCCAACAATTCTACTAACAGGAATAGGTACA
S.merriami      TAACTGGTCTAACTTAACAATTATAATAACAGGCTTAGGTACA
U.ornatus       CAACTGATCCACACCAACAATCTTATTAACAGGACTAGGCACA
U.stansburiana TAACTGATCAAACCCAACAATTTTATTAACAGGATTAGGGACA
Ancestor        CAACTGATCCYCCCCAACAATTTTATTAACAGGACTAGGCACM

C.draconoides   CTAATCACTGCAACATACTCATTACACATATTCTTAACCACCC
C.texanus       CTAATCACCGCAACCTACTCACTACATATATTCTTAACAACCC
H.maculata      CTAATTACCGCCATATACTCCCTACATATATTCTTAACCACTC
P.asio          TTAATTACAGCAATATACTCGCTACATATATTTCTAACGACCC
P.braconnieri   TTAATTACAGCAATATACTCACTACATATATTCTTAACGACCC
P.cornutum      CTAATTACAGCAATATATTCATTACACATATTCTTAATAACTC
P.coronatum     CTAATTACAGCAATATATTCACTACACATATTCTTAACAACCC
P.ditmarsi      CTAATCACAGCAATATACTCACTATACATATTCCTAACAACCC
P.douglasii     CTAATCACAGCAACATACTCACTATATATATTTTTAATAACCC
P.hernandesi    CTAATCACAGCAATATACTCACTACATATATTTTTAATAACCC
P.mcallii       CTAATTACAGCCATATATTCACTACACATATTTTTAACAACAC
P.modestum      CTAATTACAGCAATATACTCACTACATATATTTTTAATAACTC
P.orbiculare    CTAATTACAGCAATATATTCACTACACATATTTATAATAACCC
P.platyrhinos   CTAATTACAGCAATATACTCACTACACATATTTTTAACAACAC
P.solare        CTTATCACAGCAATATACTCACTACACATATTTCTAACAACCC
P.taurus        CTAATCACAGCGGCATATTCACTACATATATTTTTAACAACCC
S.merriami      CTAATCACAGCAGTATACTCCCTTCACATATTTATTATAACCC
U.ornatus       CTAATCACTGCAATATATTCACTACACATATTCCTTATAACAC
U.stansburiana CTAATCACCGCAATATATTCTCTACATATATTTTTAATAACTC
Ancestor        CTAATCACMGCAATATAYTCACTACAYATATTCTTAAYAACYC

C.draconoides   AACGAAACAAACTACCAACCCACATCCACATTATAAACCCCTC
C.texanus       AACGAAACAAACTACCAGCCCACATTCACATGCTCGACCCAAC
H.maculata      AACGAAACAAGTTACCCACTCACATCTATCTACTAGACCCAAC
P.asio          AACGAAACAAACTACCATCCCACATCTTTATACTAGACCCAAC
P.braconnieri   AACGAAACAAACCACCAACCCACATCTTTATATCTGATCCAAC
P.cornutum      AACGAAATAAACTACCAACTAACATTACTTTATCCGCTCCTAC
P.coronatum     AACGAAACAAACTCCAAACTCATATCTTTATATCTGACCCCAC
P.ditmarsi      AACGAAACAAACTACCCACCAACACCCTAATGCTTAACCCAAC
P.douglasii     AACGAAACAAACTACCAACTCACACCCTCATGTCTAACCCAAC
P.hernandesi    AGCGAAACAAACTACCAACCCACACCCTCATGTCTAACCCAAC
P.mcallii       AACGAAACAAACTACCAACCCACACCCTTATACCAAACCCCAC
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P.modestum      AGCGAAACAAACTACCAAATCACATCTTAATATTTGACCCTAC
P.orbiculare    AACGAAACAAACTACCAACCCATATCATTATCCCCAACCCAAC
P.platyrhinos   AACGAAATAAACTGCCAACCCACACCTTCATATCTAACCCCAC
P.solare        AACGAAATAAACTATCAACCCACATCTTAATTTCCGACCCCAC
P.taurus        AACGAAACAAACTACCAACCCACACCCTCATATTTGACCCCAC
S.merriami      AACGAAACAAA?TGCCAACCCATATCCAC?TACTTGATCCAAC
U.ornatus       AACGAAACAAACTAACACCTGACATCACAACAATGCACCCAAC
U.stansburiana AACGAAACAAACTCCCCACTAATATTATCCTAACCGAACCCGC
Ancestor        AACGAAACAAACTACCAACTCACATCHHCATACTHGACCCAAC

C.draconoides   ACACACACGAGAACACTTACTAATATTTCTCCACATAGCACCA
C.texanus       ACATACACGAGAGCATCTCCTAATATTTCTACACATCATACCC
H.maculata      ACACACACGAGAGCACCTACTAATATTTTTACATAT?GCACCC
P.asio          ACACACGCGAGAGCATCTACTAATAGCACTACACATGGCCCCA
P.braconnieri   ACACACACGAGAACACCTCCTAATAATACTACATACAATGCCA
P.cornutum      ACACACACGAGAACACCTACTAATAACACTGCACATAGCACCC
P.coronatum     CCATACACGAGAACACCTCCTAATAACACTACATATTTTACCA
P.ditmarsi      ACACACGCGAGAACATCTATTAATAGCGCTACACACAGCACCT
P.douglasii     GCACACACGAGAACATCTACTAATAGCACTACATACAGCACCA
P.hernandesi    GCACACGCGAGAACATCTATTAATAGCACTACATACAGCACCT
P.mcallii       ACATACACGAGAACACCTTTTAATAACCCTACATATTATACCC
P.modestum      ACACACACGAGAACATCTACTAATAATACTACATACAACACCC
P.orbiculare    ACACACGCGAGAACATCTACTAATAACATTACACATAATACCA
P.platyrhinos   ACACACACGAGAACATCTTCTAATAACACTGCATATTACCCCC
P.solare        ACACACACGAGAACATCTTCTAATAACATTACACATAGCACCC
P.taurus        ACACACGCGAGAGCACCTCCTAATAACACTACACATAACACCA
S.merriami      CCACACACGAGAACATCTT?TAATAACATTTCACCTGGCTCCA
U.ornatus       ACATACACGAGAACATCTACTAATAACA?TTCACATCATTCCA
U.stansburiana CCACACACGAGAACATCTACTAATAATACTCCATATCACCCCC
Ancestor        ACACACACGAGARCAYCTACTAATAAYAYTACACATMRCACCM

C.draconoides   CTAATTTTATTAATTATAAAACCAACACTAATTTCAGGAATTA
C.texanus       CTTATTTTATTAATTACAAAACCAACCCTAATTTCAGGAATTA
H.maculata      CTCATTTTA?TAATTATAAAACCAACACTAATCTCAGGAATTA
P.asio          TTAATTCTACTTATTATAAAACCAATGCTAATCTCAAGCGTAA
P.braconnieri   TTAATTCTACTTATTATAAAACCAACCCTGATTTCAGGTATTA
P.cornutum      TTAATTTTACTTATCATAAAACCTGCCCTAATTTCAGGCATAA
P.coronatum     TTAATTTTACTTATTATAAAACCAGCCTTAATCTCGGGCATCA
P.ditmarsi      TTACTTCTACTTATTTTAAAACCAGCCTTAATTTCAATCATCA
P.douglasii     TTACTCCTACTTATCTTAAAACCAACCTTAATTTCAGGTACCA
P.hernandesi    TTACTTCTACTCATCCTAAAACCAACCCTAATTTCAGGCATCA
P.mcallii       CTAATCTTACTTATCCTTAAACCAGCCCTAATCTCTGGCATCA
P.modestum      CTAATTTTACTTATCATAAAACCAGCCTTAATTTCAGGCATTA
P.orbiculare    CTAATTTTACTCATCATAAAACCAACCTTAATTTCAGGCATTA
P.platyrhinos   CTAATCTTACTTATTATAAAACCAACCCTAATCTCCGGCATCA
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P.solare        TTAATTCTACTCATTATAAAACCAGCCCTAATCTCCGGCATTA
P.taurus        TTAGCACTACTTATTATGAAACCAACCCTAATCTCAGGTATTA
S.merriami      CTAATCCTTTTAATTGCGAAACCGAACTTAATCTCAAACATCA
U.ornatus       TTAATCCTACTAATCACAAAACCAACACTAAT?TCGGGATTAA
U.stansburiana CTAATATTACTAATTATAAAACCGGCCCTAATTTCCGGAATTA
Ancestor        CTAATYYTACTAATTATAAAACCAACMCTAATYTCAGGAATTA

C.draconoides   TT-AATTGTTAGTATAGTTTAATAAAAACATTAGGCCGTGGCC
C.texanus       TT-GGCTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
H.maculata      TT-AACTGTTAGTATAGTTTAATAAAAACATTAGGCCGTGGCC
P.asio          TT-AACTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.braconnieri   TC-AACTGTTAGTATAGTTTAACAAAAACATTAGGCTGTGGCC
P.cornutum      TC-AACTGTTAGTATAGTTTAACAAAAACATTAGGTCGTGACC
P.coronatum     TC-AACTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.ditmarsi      TTTAACCGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.douglasii     TTTAACTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGACC
P.hernandesi    TTTAACCGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.mcallii       TT-ACCTGTTAGTATAGTTTAATAAAAACATTAGGCCGTGGCC
P.modestum      TC-AATTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.orbiculare    TT-ACCTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.platyrhinos   TT-ACTTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.solare        TT-AACTGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
P.taurus        TT-AACTGTTAGTATAGTTTAACAAAAACATTAGGCTGTGGCC
S.merriami      TT-AATTGTTAGTATAGTTTAATAAAAACATTAGGCAGTGGCC
U.ornatus       TA-TAACGTTAGTATAGTTTAACAAAAACATTAGGCCGTGGCC
U.stansburiana TC-AACTGTCAGCATAGTTTAATAAAAACATTAGGCCGTGGCC
Ancestor        TTTAACTGTTAGTATAGTTTAATAAAAACATTAGGCCGTGGCC

C.draconoides   CTAAAAACAGAAGATTACCCCTTCTTACAAACCAAGAGGTGCT
C.texanus       CTAAAAACAGAAGTTTACCCCTTCTTACAAACCAAGAGGTGTT
H.maculata      CTAAAAACAGAAGTTCACCCCTTCTTACAAACCAAGAGGTGCT
P.asio          CTAAAAACAGAAGATCACCTCTTCTTACAAACCAAGAGGTGTT
P.braconnieri   CTAAAAATAGAAGATCGCCTCTTCTTGCAAACCAAGGGGTGTT
P.cornutum      CTAAAAATAGAAGCTCATCCCTTCTTACAAACCAAGAGGTGTT
P.coronatum     CTAAAAACAGAAGCTCACCCCTTCTTACAAACCAAGAGGTGTT
P.ditmarsi      CTAAAAATAGAAGCTCATAACTTCTTACAAACCAAGGGGTGTT
P.douglasii     CTAAAAACAGAAGCTCATAACTTCTTACAAACCAAGAGGTGTT
P.hernandesi    CTAAAAACAGAAGCTCATAACTTCTTACGAACCAAGAGGTGTT
P.mcallii       CTAAAAATAGAAGCTTATTACTTCTTACAAACCAAGGGGTGTT
P.modestum      CTAAAAACAGAAGCTCACCCCTTCTTACAAACCAAGAGGTGTT
P.orbiculare    CTAAAAACAGAAGCTAACATCTTCTTACAAACCAAGGGGTGTT
P.platyrhinos   CTAAAAATAGAAGTTTATTTCTTCTTACAAACCAAGGGGTGTT
P.solare        CTAAAAACAGAAGCTAAATACTTCTTACAAACCAAGAGGTGTA
P.taurus        CTAAAAATAGAAGCTCGCCTCTTCTTACAG?CCAAGAGGTGTT
S.merriami      CTAAAAACAGAAGTTAAATCCTTCTTACAAACCAAGGGGTGTT
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U.ornatus      CTAAAAACAGAAGCTTAAAA?TT?TTACAA?CCAAGAGGTGTA
U.stansburiana CTAAAAACAGAAGATAACACCTTCTTACAGACCAA--------
Ancestor       CTAAAAACAGAAGTTTAMMCCTTCTTACAAACCAAGAGGTGTT

C.draconoides  ????????????????????????????????
C.texanus      ????????????????????????????????
H.maculata     ????????????????????????????????
P.asio         00000120001200002000102012001200
P.braconnieri  020001{02}3111011000000002012120300
P.cornutum     00412112120210000001211011000310
P.coronatum    004021121202000311000100100001{23}01
P.ditmarsi     01100120021000002000003012101301
P.douglasii    ????????????????????????????????
P.hernandesi   00202101120001022100000010101{23}01
P.mcallii      20411112120110110111211210020301
P.modestum     10311102120010100111011100010311
P.orbiculare   00202001120001022100000010101301
P.platyrhinos  003111{01}21200101101111011110010301
P.solare       00410112120010200110211010021401
P.taurus       02000123111011000000202012110100
S.merriami     ????????????????????????????????
U.ornatus      ????????????????????????????????
U.stansburiana ????????????????????????????????
Ancestor       0000?000000000?00000000000000000
     ;
End;

BEGIN ASSUMPTIONS;
OPTIONS DEFTYPE=unord PolyTcount=MINSTEPS;
EXSET * UNTITLED = 121-125 187-193 430-473 535-537;
TYPESET * UNTITLED = unord: 1-2510 2513-2519 2521 2523-2526
2528-2530 2532-2535
2537 2539-2542, ord: 2511-2512 2520 2522 2527 2531 2536
2538;
WTSET * UNTITLED = 2: 1-2510 2512-2526 2528-2530 2532-2533
2537-2542, 1: 2511 2527 2531 2534-2536;
ENDBLOCK;
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APPENDIX 3: SPECIMENS EXAMINED

Specimens examined for Chapter 2. Abbreviations found on specimen identifications refer to the collections below. After the abbreviations is a list of curators and collection managers contacted for the various specimens. Dissections of the following specimens were made for analyzing reproductive condition and additional museum record information (e.g. locality data) were obtained and used from additional undissected specimens also contained in the specimen list.

Abbreviations:
BMNH: British Museum Natural History
CB: Escuela Nacional de Ciencias Biologicas, Instituto Politecnico Nacional
CAS: California Academy of Sciences
EBS: Elizabeth Beltran Sanchez, personal collection, Instituto de Investigaciones Científicas Area de Ciencias Naturales, Universidad Autónoma Guerrero
EB-UAP: Laboratorio de Herpetología Escuela de Biología Benemérita Universidad Autónoma de Puebla
IBH: Instituto Biologia Herpetologica, Coleccion Nacional de Anfibios y Reptiles, Universidad Nacional Autónoma de Mexico.
KU: Kansas University
LACM: Los Angeles County Museum
MCZ: Museum of Comparative Zoology
MFO: Manuel Feria Ortiz, personal collection
MZFC: Museo Zoologia Facultad de Ciencias, Universidad Nacional Autónoma de Mexico.
SDSNH: San Diego State Natural History
USNM: United States National Museum
UTA: University of Texas at Arlington
WLH, EPR, HRG: Wendy L. Hodges, personal collection, final deposition of all specimens will be in the MZFC

Museum Contacts:
Adrian Nieto Montes de Oca, Oscar Flores Villela, Edmundo Perez Ramos (MZFC)
Victor Hugo Reynoso (IBH),
Luis Conseco Marquez (EB-UAP)
Fernando Mendoza Quijano, Elizabeth Beltran Sanchez (EBS, personal collection)
Manuel Feria Ortiz (MFO, personal collection)
David Kizirian (LACM)
John Simmons (KU)
Colin McCarthy (BMNH)
Brad Hollingsworth (SDSNH)
Jose P. Rosado (MCZ)
Kevin de Queiroz (USNM)
J. Vindum (CAS)
Carol Stewart and Jonathan Campbell (UTA)
Ticul Alvarez and Sergio Murillo (CB)

P. asio: AMNH62607, AMNH68135, AMNH68137, AMNH68139, CB2005, CB3421, CB574, EBS-066, EPR1097, EPR1099, EPR1105, EPR1115, EPR1116, EPR1132, EPR1137, EPR1138, FMNH105256, FMNH105257, FMNH108070, FMNH108072, FMNH108077, FMNH108079, FMNH108081, FMNH39470, FMNH40826, FMNH40845, FMNH40846, FMNH72429, HRG101, HRG102, HRG103, HRG109, HRG110, HRG114, HRG115, HRG116, HRG117, HRG118, HRG119, HRG120, HRG121, HRG124, HRG125, HRG126, HRG127, HRG128, HRG129, HRG130, HRG131, HRG132, HRG133, HRG134, HRG136, IBH0025, IBH0096, IBH0845, IBH1166, IBH1669, IBH2774, IBH2925, IBH3322, IBH4175, IBH5610, IBH5681, IBH612, IBH6474, IBH7478, IBH777, IBH782, IBH8221, IBH9433, IBH-DBN0053, IBH-DBNoo37, KU37763, KU40388, KU40389, KU61484, LACM37620, LACM6617, MZFC9507, SDSNH19711, SDSNH41193, SDSNH41194, SDSNH41195, SDSNH41196, SDSNH42066, SDSNH42067, WLH1033, WLH1036, WLH1037, WLH1038, WLH1039, WLH1040, WLH1041, WLH1043, WLH1051, WLH1052, WLH1056, WLH1078, WLH1082, WLH1083, WLH1087, WLH1088, WLH1089, WLH1090, WLH1091, WLH1093, WLH1094, WLH1095, WLH1097, WLH1144/pa 01, WLH1145/pa 02, WLH1146/pa 03, WLH1147/pa 04, WLH1148/pa 05, WLH1149/pa 06, WLH1150/pa 07, WLH1151/pa 08, WLH1152/pa 09, WLH1153/pa 10.

P. braconnieri: AMNH100731, AMNH106817, AMNH106820, AMNH89669, AMNH89670, AMNH98086, EB-UAP24, EB-UAP742, EB-UAP743, EB-UAP76, FMNH106208, KU37761, MZFC0174, UTA11391, UTA11392, UTA11393,

UTA25790, UTA25791, UTA4222, UTA4348, UTA4588, UTA4594, UTA4837, UTA4838, UTA4839, UTA4840, UTA7732, WLH1111, WLH1112, WLH1117, WLH1118, WLH1119, WLH1120.

P. ditmarsi: LACM140238, WLH1012, WLH1013, WLH1125, WLH1126, WLH1127, WLH1128, WLH1131, WLH1132, WLH1134, WLH1135, WLH1136, WLH1138, WLH1139, WLH1140, WLH1141.

P. orbiculare: AMNH15423, AMNH15424, AMNH15427, AMNH18482, AMNH537, AMNH62319, AMNH62322, AMNH69702, AMNH72410, AMNH73783, AMNH75818, AMNH75866, AMNH77534, CB10610, CB3214, CB3215, CB3216, CB3217, CB4153, CB4154, CB4155, CB4178, CB8061, CB8062, FMNH102375, FMNH102376, FMNH102377, FMNH177362, FMNH70783, FMNH70784, FMNH70785, IBH02924-2, IBH0313, IBH0683, IBH0777,1-17, IBH0781, IBH0782, IBH1022, IBH1129, IBH1498, IBH1663, IBH1670, IBH2249, IBH2402, IBH2924, IBH3169, IBH3309, IBH3625, IBH3642, IBH3708, IBH4660, IBH5759, IBH6155, IBH9434, IBH9435, KU105706, KU23746, KU23747, KU23748, KU25852, KU25853, KU25854, KU25855, KU25856, KU25857, KU25859, KU25860, KU25861, KU25862, KU25863, KU25864, KU25865, KU25866, KU25867, KU25868, KU25869, KU25870, KU25871, KU25872, KU25873, KU25874, KU25875, KU25876, KU25877, KU25878, KU25879, KU25880, KU25881, KU25882, KU25883, KU25884, KU25885, KU25886, KU28067, KU29799, KU40386, KU40387, KU44163, KU44164, KU44165, KU44166, KU51764, KU56211, KU61502, KU61503, KU92571, LACM125319, LACM1816, LACM1817, LACM36698, LACM36699, MFO-040, MFO-041, MFO-A51Fogoxos 007, MZFC00229, MZFC00230, MZFC00602, MZFC0172, MZFC0231, MZFC0337, MZFC0622, MZFC0784, MZFC08471, MZFC08472, MZFC2320, MZFC2379, MZFC3104, MZFC3243, MZFC3243-2, MZFC3244, MZFC3287, MZFC3530, MZFC4698, MZFC6245, MZFC6337, MZFC6337-3, MZFC7383, MZFC-EAM 074, MZFC-EAM 35, MZFC-EAM 47, MZFC-EAM032, MZFC-EAM037, MZFC-EAM066, WLH01020, WLH01021, WLH01026.

P. taurus: CB13626, EB-UAP 1116, EB-UAP 1117, EB-UAP 1118, EB-UAP 430, FMNH105464, HRG104, HRG111, HRG135, KU37802, MFO/JmmV127, MFO151, MZFC 0149, MZFC 0173, MZFC 5861, MZFC/ACS 002, WLH1029, WLH1030, WLH1031, WLH1081, WLH1084, WLH1086, WLH1096, WLH1099, WLH1100, WLH1101, WLH1102, WLH1103, WLH1104, WLH1105, WLH1106, WLH1107, WLH1108, WLH1109, WLH1110, WLH1121, WLH1122, WLH1123, WLH1124.

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